

**Induction of resistance in rice to white backed plant hopper, *Sogatella furcifera* (Horvath) through application of zinc**  
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# **Induction of resistance in rice to white backed plant hopper, *Sogatella furcifera* (Horvath) through application of zinc**

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COLLEGE OF AGRICULTURE  
ODISHA UNIVERSITY OF AGRICULTURE AND  
TECHNOLOGY  
BHUBANESWAR-751003, ODISHA  
2019**

**Induction of resistance in rice to white backed  
plant hopper, *Sogatella furcifera* (Horvath)  
through application of zinc**

*A Thesis submitted to the Odisha University of Agriculture and Technology  
in Partial fulfilment of the Requirements for the degree of Doctor of  
Philosophy in Agriculture (Entomology)*

**By**

***Seema Tripathy***

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Date:

## **CERTIFICATE-I**

This is to certify that the thesis entitled “**Induction of resistance in rice to white backed plant hopper, *Sogatella furcifera* (Horvath) through application of zinc**” submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Agriculture (Entomology)** to the **Odisha University of Agriculture and Technology** is a faithful record of bonafide and original research work carried out by **Seema Tripathy, Adm. No. 03ENT/Ph.D/15** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged

**CHAIRMAN  
ADVISORY COMMITTEE**



## CERTIFICATE-II

This is to certify that the thesis entitled “**Induction of resistance in rice to white backed plant hopper, *Sogatella furcifera* (Horvath) through application of zinc**” submitted by **Seema Tripathy** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **Doctor of Philosophy in Agriculture (Entomology)** has been approved/disapproved by the students’ advisory committee and the external examiner.

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**Place: Bhubaneswar**

**Date:**

**Seema Tripathy**

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## LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Percentage
µg	:	Microgram
µl	:	Microlitre
µM	:	Micromolar
°C	:	Degree Celsius
CD	:	Critical difference
cm	:	Centimetre
CRBD	:	Completely Randomized Block Design
DAS	:	Days after Sowing
DAT	:	Days after transplanting
EDTA	:	Ethylene diamine tetra-acetic acid
et al.	:	etalebi (and others)
etc.	:	et cetera
Fig.	:	Figure
g	:	Gram
ha	:	Hectare
i.e.	:	That is
kDa	:	kilodalton
kg	:	Kilogram
m	:	Meter
M	:	Molarity
mM	:	Milimolar
mg	:	Milligram
min	:	Minute
ml	:	Milliliter
N	:	Normality
nm	:	Nanometer
PAL	:	Phenylalanine ammonia lyase
POD	:	Peroxidase
pH	:	Hydrogen concentration
ppm	:	Parts per million
q	:	Quintal
r	:	Correlation coefficient
ROS	:	Reactive oxygen species
S	:	Significant
SE <sub>(m)</sub> ±	:	Standard error of mean
SOD	:	Superoxide dismutase
viz.	:	Namely
WBPH	:	White backed plant hopper

## ABSTRACT

Induction of resistance in rice to white backed plant hopper (WBPH) through application of zinc was conducted at the Central Research Farm, Department of Entomology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar through a series of field and pot culture experiments during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017. It was revealed that the incidence of WBPH was more during *kharif* season than summer season. Application of zinc in rice caused induced antixenosis effect in rice to WBPH through reduced nymphal and adult orientation and also caused reduced oviposition as compared to no treatment (control). Induction of antibiosis mechanism following zinc application in rice was also spectacular. Zinc treated rice plants caused reduced nymphal survival, increased nymphal duration, lower growth index, less feeding efficiency, lower population build up and increased male formation in WBPH. Zinc application reduced the functional plant loss index and enhanced the wilting period as compared to control plants indicating the development of an induced tolerance mechanism in the zinc treated plants. Zinc application to rice plants favoured more chlorophyll production, enhanced plant growth and yield attributing parameters. Various biochemical constituents like total phenol and total soluble protein content were increased due to zinc application, whereas, total free amino acid, total soluble sugar and proline content were reduced. Various enzymes like superoxide dismutase (SOD), peroxidase (POD) and phenylalanine ammonia lyase (PAL) were triggered in zinc applied plants than control. The total chlorophyll, total phenol, total soluble protein, SOD, POD and PAL activity were significant and negatively correlated with WBPH population, whereas, total free amino acid, total soluble sugar and proline content were positively correlated to WBPH population. Protein profiling studies revealed that various combined application of ZnSO<sub>4</sub> and Zn EDTA produced specific polypeptide bands of 66.0 kDa, 37.0 kDa and 14.3 kDa, which was not seen in control. Since, the polypeptide band 23.6 kDa was synthesized in T<sub>6</sub> (Zn EDTA basal @ 40 kg/ha + Zn EDTA foliar spray @ 0.8 % twice at 30 and 45 days after transplanting (DAT)) and T<sub>7</sub> (ZnSO<sub>4</sub> basal @ 25kg/ha + Zn EDTA foliar spray @ 0.8% twice at 30 and 45 DAT) which supported least WBPH population, the above protein i.e. 23.6 kDa was adjudged as most defensive protein responsible for induced resistance in rice plant to WBPH.

# INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple food crop in the world, which provides over 21 per cent of the dietary calories for more than half of the global world population (Fitzgerald *et al.*, 2009). It is rightly said that ‘Rice is life’ because of its nutritional quality. This is the most important food crop in terms of area, production and consumer preference which along with wheat was instrumental for success of green revolution in India. Globally, India has largest area under rice cultivation i.e. about 43.99 million hectares and stands second with an annual production of 109.7 million tonnes (Directorate of Economics and Statistics, 2018). India demand for rice by the year 2050 is estimated to be about 137.3 million tonnes as compared to the present production status (CRRI, 2013). With the negligible scope for expansion of area, the growth rate of rice production must not only be sustained but should be accelerated to meet the food demand of burgeoning population. One of the major impediments in meeting this demand is yield loss due to biotic and abiotic stresses. About 52 per cent of the global rice production is lost annually by various biotic factors, out of which approximately 21 per cent damage is caused due to the attack of insect pests (Yarasi *et al.*, 2008).

More than 100 species of insects have been reported to attack this crop (Krishanaiah *et al.*, 2008). Out of these, about 20 species are of major economic significance. Three major sap-sucking pests of rice *viz.*, brown plant hopper, *Nilaparvata lugens* (Stal), white backed plant hopper, *Sogatella furcifera* (Horvath) and green leaf hopper, *Nephotettix virescens* (Distant) are of economic concern in India and known to cause severe damage to rice plants (Foissac *et al.*, 2000). The white backed plant hopper (WBPH) primarily feeds on rice plants and can migrate over long distances in the temperate and tropical regions of Asia and Australia (Mun *et al.*, 1999). Serious damage usually occurs during the early stages of plant growth with symptoms of hopper burn due to intensive sucking by WBPH (Dale, 1994). Under favourable conditions, it can cause 35-95 per cent yield loss (Sidhu, 1979). It is responsible for destruction of approximately 10 million hectares of rice crops annually in China (Cheng, 2009). More importantly, the WBPH transmits devastating rice virus, i.e. southern rice black-streaked dwarf virus, which poses an additional threat to rice plants (Zhou *et al.*, 2013).

Insecticide misuse, cultivation of high yielding rice varieties, cultural and climatic factors, long-distance migration ability and high fecundity of WBPH together results in outbreaks of this pest (Zhai *et al.*, 2013). Development of pesticide resistance in insects particularly in plant hoppers has become a major problem due to indiscriminate use of pesticides. One of the principal factors contributing to the increase in severity of this insect is the indiscriminate and uninhibited use of insecticides, which also kill many natural enemies (Balasubramanian *et al.*, 1988). With the advent of chemical pesticides, the crisis was resolved to a greater extent.

Even though rice production in recent years have been increased, yet, certain agronomical and pedological attention has to be necessitated for improving the physiological condition of the rice plants to keep them fit to resist diseases and insect pest infestations, thereby, improving rice yield. Unfortunately, no truly resistant varieties have also been developed in rice against this insect so far. Thus, the alternate non-conventional and eco-friendly approach for protection of plant for sustainable agriculture is to manage this pest through induction of resistance in the rice through application of various micronutrients.

Induced resistance is a defense system within the plants which allows them to resist attacks from pests such as bacterial or fungal pathogens or insects. The defense system reacts to the external attack with physiological changes triggered by the production of proteins and chemicals that lead to activation of the plant's immune system. Induced resistance occurs in the plant following a stimuli caused by elicitors. There are two types of elicitors i.e. biotic and abiotic. Elicitors of biotic origin include beneficial microbes, insects, botanicals and other organic products. Elicitor of abiotic origin includes chemical elicitors, herbicides, fertilizer and mineral nutrients (e.g. zinc, silicon, iron fertilizers etc.). Thus, one or both the elicitors produce a stimuli in the plants as a result of which certain signal transduction occurs and defensive pathways are triggered producing substances which impair growth and development of the attacking organisms.

Zinc deficiency is a widespread problem in rice grown under flooded conditions, limiting growth and grain zinc accumulation. Zinc is generally taken up as free divalent cation ( $Zn^{2+}$ ), but it may be absorbed as monovalent cation ( $ZnOH^+$ ) at high pH. Zinc is an essential micronutrient for the normal healthy growth and

reproduction of plants and when the supply of plant available zinc is inadequate, crop yields are reduced and the quality of crop products are impaired. In plants, zinc plays a key role as a structural constituent or regulatory cofactor of a wide range of different enzymes and proteins in many important biochemical pathways and these are mainly concerned with carbohydrate metabolism, protein metabolism, auxin production, pollen formation, maintenance of the integrity of biological membranes and resistance to infection by certain pathogens (Alloway, 2004). Srivastava and Gupta (1996) reported that zinc is a structural part of carbonic anhydrase, alcohol dehydrogenase, Cu/Zn-superoxide dismutase and RNA polymerase as well as serves as a cofactor for all 6 classes of enzymes (oxidoreductases, transferases, hydrolases, lyases, isomerase and ligases).

Salicylic acid (SA), a benzoic acid derivative, is an important endogenous plant growth regulator that generates a wide range of metabolic and physiological responses in plants involved in defense in addition to their impact on plant growth and development (Vicente and Plasencia, 2011). SA induces greater defense against piercing and sucking type of insect pests than the chewing ones (Zhao *et al.*, 2009). SA signalling molecule is associated with local defense as well as induction of systemic resistance. Chen *et al.* (2009) have suggested that plants synthesize SA from cinnamate through phenylpropanoid pathway by the activity of phenylalanine ammonia lyase (PAL) and Wadhwa *et al.* (2014) reported that zinc acts as a cofactor for the synthesis of PAL enzyme. Production of reactive oxygen species ( $H_2O_2$ ) by SA pathway defends the plant against various insect pests since;  $H_2O_2$  actively damages the digestive system of insects leading to reduced growth and development (Maffei *et al.*, 2007). Hence, though there is no direct evidence that zinc is responsible for synthesis of SA, yet, the above narration speaks of indirect role of zinc in SA synthesis.

PAL also catalyses the biosynthesis of phenolics which have an important role in insect resistance mechanism (Punithavalli *et al.*, 2013). Southerton (1990) also observed direct correlation of induction of PAL activity with phenol content. Oxidation of phenols catalysed by polyphenol oxidase (PPO) and peroxidase (POD) is a major defense mechanism in plants against herbivorous insects and Quinones formed by the oxidation of phenols bind covalently to leaf proteins and inhibit the protein digestion in herbivores. Alkylation of amino acids reduces the nutritional value of plant proteins for insects, which in turn negatively affects the growth and development of insect.

Wadhwa *et al.* (2014) have reported that application of  $Zn^{2+}$  ( $20 \text{ mg kg}^{-1}$ ) as soil treatment induced high activity of the antioxidative enzymes namely POD and PPO in cluster bean.

The amount of zinc in unpolluted soils typically are lower than 125 ppm (Hussain *et al.*, 2010) and in plants growing in these soils this metal concentration varies between  $0.02 - 0.04 \text{ mg g}^{-1}$  dry weight (Bowen, 1979). Zinc is an essential micronutrient involved in a wide variety of physiological processes (Broadley *et al.*, 2007), yet, at concentrations above  $0.2 \text{ mg g}^{-1}$  dry matter the potential phytotoxicity at leaf tissue develops (Ali *et al.*, 2000). Alam and Kumar (2015) reported that various vegetative growth parameters of rice like plant height, plant dry weight, number of tillers /  $\text{m}^2$ , number of effective tillers /  $\text{m}^2$ , crop growth rate ( $\text{g} / \text{m}^2 / \text{day}$ ), relative growth rate ( $\text{g} / \text{g} / \text{day}$ ) as well as yield attributing traits like length of panicle, number of grains per panicle, number of filled grains per panicle, test weight, grain yield and straw yield were increased by external supplementation of zinc to rice plant.

Few investigations have also been conducted to evaluate the role of zinc in the management of WBPH in rice. Pati (2002) studied the effect of zinc in suppressing WBPH in rice. Rath (2004) have also reported that zinc affected the growth and development of rice WBPH causing lower nymphal survival, prolonged nymphal duration, lower female: male ratio, lower growth index and population build up and exercised a possible induced antibiosis in rice against WBPH.

Keeping the aforesaid consideration in view, the present studies were taken up with the following objectives:

- i) Evaluation of the effect of zinc application on the incidence of WBPH in rice under field condition
- ii) Assessment of the mechanism of resistance (antixenosis, antibiosis and tolerance) against WBPH in rice plant through zinc uptake under pot culture experiment
- iii) Determination of zinc induced biochemical changes of rice plant at maximum incidence of WBPH
- iv) Working out the correlation between zinc content vs. various plant growth attributes, biochemical parameters and WBPH population
- v) Characterization of zinc induced defensive protein in rice to WBPH through qualitative analysis

# REVIEW OF LITERATURE

The present investigation on “Induction of resistance in rice to white backed plant hopper, *Sogatella furcifera* (Horvath) through application of zinc” was thought of to ascertain the induced effect of zinc in rice plant against the white backed plant hopper. Various literature pertaining to the present study is critically reviewed in this chapter.

## 2.1 Economic importance of white backed plant hopper in rice

The white backed plant hopper (WBPH) was first reported to cause very heavy damage on rice from Kapurthala, Ludhiana, Karnal, Gurgaon, Sangrur and Ambala district of joint Punjab in 1966 (Atwal *et al.*, 1967). Sidhu (1979) has observed that a population density of 400-500 WBPH nymphs or 200 adults per plant could cause total crop loss in rice.

In China, WBPH was considered as a minor insect pest of rice before the 1970s. But from the report of Tan (1987) from China, it was indicated that after release of hybrid rice in 1976, WBPH outbreak happened on hybrid rice Nanyou 2 in Hunan Province in 1977. In 1982, about 1,600 ha of Shanyou 6 fields were severely infested with WBPH, and 80 ha were completely destroyed in the hybrid rice pilot areas in Guangdong Province, South China, where Shanyou 2, Shanyou 6 and Weiyou 6 were introduced deliberately (Feng and Huang, 1983). Liu *et al.* (2000) also stated that *Sogatella furcifera* has emerged as an important pest on rice in China and South East Asian countries.

Saini (1984) has observed complete hopper burn of paddy over 1000 ha due to high population density of WBPH (200-500 insects/hill) in Punjab. Khan and Saxena (1986) described WBPH as one of the major pests of rice which was responsible for severe yield losses. Prakash and Rao (1998) estimated 11-39 per cent yield loss in rice due to WBPH attack. The yield loss was attributable to decrease in leaf area, plant height, dry weight, leaf and stem nitrogen concentration, chlorophyll contents and photosynthetic rate (Watanabe and Kitagawa, 2000).

Krishnaiah and his co-workers in 2008 have stated that WBPH had emerged as a serious pest in areas particularly where rice varieties resistant to brown plant hopper

(BPH) were grown. In India, by 2008 extensive damage to rice crop in many states like Punjab, Haryana, Madhya Pradesh, Uttar Pradesh, Orissa, Andhra Pradesh, Kerala, Puducherry, Tamil Nadu and Assam due to WBPH was observed (Alice and Sujetha, 2008). Recently, WBPH has been considered as the principal species in plant hopper assemblages and caused severe loss in rice in India and particularly in East Asia (Sogawa *et al.*, 2009).

In 2013, Zhou and his associates studied that the WBPH is considered as a serious pest of rice in Asia which damaged rice directly by feeding on the rice or indirectly by transmitting plant viruses such as southern rice black-streaked dwarf virus. The transmission of viral disease by WBPH is of recent finding in China.

## **2.2 Importance of zinc in plants**

Zinc is one of the most important micronutrients for plants and also a constituent of carbonic anhydrase and it is required for the activity of ribulose 1, 5-bisphosphate carboxylase/ oxygenase (Rubisco), the photosynthetic enzymes catalysing the diffusion of CO<sub>2</sub> to the chloroplasts through the cell (Hatch and Slack, 1970). In the year 1984, Tisdale and his co-workers suggested that zinc plays a central role in plant metabolism by influencing the activities of carbonic anhydrase and hydrogenase, stabilization of ribosomal fractions and synthesis of cytochrome. Zinc is also involved in maintaining the structural and functional integrity of biological membranes (Sadeghzadeh and Rengel, 2011) mainly due to its binding to sulfhydryl containing compounds (Willson, 1988).

In the year 1998, Fox and Guerinot ascertained that zinc plays a key role as a structural constituent or regulatory co-factor for more than 300 enzymes involved in many important biochemical pathways. Zinc is a structural component of many transcription factors as well as various enzymes *viz.*, carbonic anhydrase, alcohol dehydrogenase, Cu/Zn-superoxide dismutase and RNA polymerase and it is the only metal ion that serves as a co-factor for all the six classes of enzymes (oxidoreductases, transferases, hydrolases, lyases, isomerase and ligases) (Auld, 2001 and Gupta *et al.*, 2016).

Plant enzymes which get activated by zinc are mainly involved in carbohydrate metabolism (both in photosynthesis and in the conversion of sugar to starch), maintenance of the integrity of cellular membranes, protein synthesis, regulation of

auxin synthesis, pollen formation and the resistance to infection by certain pathogens (Alloway, 2004). Zinc is also found to be involved in signal transduction via mitogen-activated protein kinases (Lin *et al.*, 2005).

In plants, zinc has many roles including membrane function, photosynthesis, gene expression, protection against drought and pathogens as well as for the synthesis of hormones that are involved in plant growth and development. Zinc is found at high levels of accumulation in seeds, indicating its importance in fertility (Hefferon, 2019).

Zinc deficiency results in the development of abnormalities in plants which become visible as deficiency symptoms such as stunted growth, chlorosis, smaller leaves and spikelet sterility. Zinc deficiency can also adversely affect the quality of harvested products; plants susceptibility to abiotic stress and fungal infection (Cakmak, 2000). The physiological stress caused by zinc deficiency resulted in development of abnormalities in plants and hence, zinc is found to be important for proper growth and development of plants (Roohani *et al.*, 2013).

## **2.3 Role of zinc in rice plant**

### **2.3.1 Effect of zinc on growth attributes (height and tiller number) of rice plant**

Shivay *et al.* (2008) conducted an experiment to study the effect of various concentrations of zinc enrichment of urea in aromatic rice-wheat cropping system for two successive years and reported that application of 3.5 per cent zinc enriched urea recorded the highest plant height (105.2 cm) of rice plant. Shivay *et al.* (2010) also reported that with the incremental dose of zinc, the plant height of aromatic rice cultivars was found to increase and with the application of 7.5 kg Zn ha<sup>-1</sup>, the plant height (103.1 cm) was found to be highest.

Increase in plant height of rice due to zinc application has been witnessed by Singh *et al.* (2012). Alam and Kumar (2015) also observed a similar biological event. However, Sudha and Stalin (2015) observed an increase in plant height at maturity stage in the treatment consisting of NPK with zinc application than that of control and he reported that zinc application significantly increased the plant height which might be attributed to the adequate supply of zinc that perhaps contributed to accelerate the enzymatic activity and auxin metabolism in rice plants. These results are in agreement with the statements of Khan *et al.* (2007) and Ghoneim (2016).

Jatav and Singh (2018) reported that in rice plant, maximum height (6.62% increase over control) was observed in T<sub>7</sub> (5.0 kg Zn ha<sup>-1</sup> soil application + 0.5% ZnSO<sub>4</sub> foliar spray + 0.25 % lime foliar spray at tillering and milking stage) in which the maturity of grains was delayed and height was considerably more because of adequate supply of zinc.

Kumari *et al.* (2019) reported that rice plants fertilized with soil application of ZnSO<sub>4</sub> @ 37.5 kg/ha showed maximum height (109.82 cm) at harvest as compared to other zinc fertilization treatments which was significantly superior over control but was statistically at par with soil application of ZnSO<sub>4</sub> @ 25 kg/ha and foliar application of ZnSO<sub>4</sub> @ 0.5 per cent at tillering, pre-flowering and flowering stage. This might be due to adequate supply of zinc that triggered to synthesize auxin in plants, and as auxin promote cell enlargement resulting in elongation of coleoptile, stem etc. thus, resulted in higher plant height. Yadi *et al.* (2012), Muamba and Ambara (2013) and Sudha and Stalin (2015) have also observed similar phenomenon.

Khan *et al.* (2007) described that the zinc treatments increased the number of tillers per rice hill significantly compared to control. They further opined that the increase in tillering might be attributed to zinc influencing improved enzymatic activity and auxin metabolism in plants.

Alam and Kumar (2015) revealed that zinc application in rice promoted plant growth through increase in total tiller number. Ghoneim (2016) had also observed increased tiller number by soil application of zinc which he attributed to increase in availability of nutrients in soil.

Kumari *et al.* (2019) reported that soil application of ZnSO<sub>4</sub> @ 37.5 kg/ha produced more number of tillers/m<sup>2</sup> (357.06) as compared to other treatments at 60 days after sowing (DAS) which was significantly superior over control but was statistically at par with soil application of ZnSO<sub>4</sub> @ 25 kg/ha and foliar application of ZnSO<sub>4</sub> @ 0.5 per cent at tillering, pre-flowering and flowering, respectively.

### **2.3.2 Effect of zinc on physiological parameter (chlorophyll content) of rice plant**

Aravind and Prasad (2004) indicated that zinc is involved in synthesis of chlorophyll through nutrient regulated homeostasis in cytoplasm. Zinc plays a vital role

in triggering some of the enzymes related to chlorophyll biosynthetic pathway (Ayad *et al.*, 2010). Mousavi (2011) also reported that foliar or soil application of zinc increased the biosynthesis of chlorophyll, which was important for the photosynthetic process. Zinc fertilization resulted in considerable increase in the chlorophyll content of rice leaves (Mathpal *et al.*, 2015).

Kumari *et al.* (2019) studied that Soil Plant Analysis Development (SPAD) value was significantly influenced because of zinc application. Higher SPAD value (39.21) was recorded with the soil application of ZnSO<sub>4</sub> (37.5 kg/ha) at 60 DAS which was superior over control and foliar application of ZnSO<sub>4</sub> (0.5 %) at tillering, pre-flowering and flowering stages. The same treatment was statistically at par with soil application of ZnSO<sub>4</sub> (25 kg/ha). Mumba and Ambara (2013) also studied that zinc application improved photosynthetic activity through chlorophyll formation.

### **2.3.3 Effect of zinc on yield and yield attributes of rice plant**

Application of zinc in rice caused increase in panicle length which has been well documented by Maqsood *et al.* (1999). Sudha and Stalin (2015) have also studied a similar effect in rice due to zinc application, wherein the authors recorded a 6 per cent extra length of the panicles containing more grains irrespective of the genotypes tested.

Rathore *et al.* (2004) reported that 1000-grain weight was more with the application of 180 kg N, 80 kg each of P and K + 0.5 % Zn foliar application + FYM @ 10 t ha<sup>-1</sup> on silty loam soils of Pantnagar, Uttar Pradesh. Shivay *et al.* (2010) reported that application of Zn @ 7.5 kg ha<sup>-1</sup> enhanced the yield attributes like panicles hill<sup>-1</sup> (9.7), panicle length (27.3 cm) and grains panicle<sup>-1</sup> (113.4) in aromatic rice varieties when compared to control. Yadi *et al.* (2012) reported that zinc application significantly increased the filled grains per panicle (68 to 199) over control, which clearly depicted a clear cut transport of zinc augmented food material from source to sink.

Ghasal *et al.* (2015) studied the effect of zinc fertilization on aromatic rice, Pusa Rice Hybrid 10 and reported that application of 1.25 kg Zn / ha (Zn-EDTA) + 0.5 per cent foliar spray at maximum tillering and panicle initiation stages produced highest dry matter accumulation, panicle length and grain weight (g) per panicle leading to the highest grain yield (5.67 t/ha). The highest number of panicle m<sup>-2</sup> (350), number of spikelets panicle<sup>-1</sup> (129), filled grains (82 %) and 1000 grain weight (27.1 g)

were recorded with the soil application of 15 kg Zn ha<sup>-1</sup> just after transplanting in rice (Ghoneim, 2016).

Application of Zn @ 7.5 kg ha<sup>-1</sup> in rice resulted in highest number of productive tillers per square meter, number of filled grains per panicle, test weight, grain yield and straw yield (Silviya and Stalin, 2017). The per cent increase in above parameters was 18.3, 4.4, 2.8, 29.2 and 25.0 per cent, respectively over control (NPK alone).

Firdous *et al.* (2018) conducted a field study and observed that application of zinc to soil @ 5 kg/ha in addition to two foliar sprays (0.5% at tillering and before flowering) produced significant impact on the grain yield in rice and its components i.e. panicle length, number of spike per panicle, number of grains per spike, 1000 grain weight and sterility percentage etc.

Jatav and Singh (2018) stated that maximum number of panicles per hill (5.87) was produced by application of recommended dose of fertilizer along with 0.5 per cent ZnSO<sub>4</sub> and 0.25 per cent lime as foliar spray at tillering and milking stage. Kumari *et al.* (2019) reported that among the zinc treatments, maximum panicle length (24.29 cm) was recorded with soil application of ZnSO<sub>4</sub> @ 37.5 kg/ha which was significantly superior over control but was at par with soil application of ZnSO<sub>4</sub> @ 25 kg/ha and foliar application of ZnSO<sub>4</sub> @ 0.5 per cent at tillering, pre-flowering and flowering stages.

## **2.4 Zinc induced resistance to rice pests**

### **2.4.1 Antixenosis mechanism**

In resistant variety, antixenosis was suggested to be more because of gustatory stimuli rather than olfactory or visual influence (Pathak and Saxena, 1980). Though morphological characters may influence the alighting response of the hoppers, they were not considered as main source of non-preference because the hoppers could differentiate the resistant and susceptible varieties, when the plants were morphologically similar (Gunathilagaraj and Chelliah, 1985). The BPH showed no significant preference in landing on different varieties, but the insect did not stay on resistant line for continuous feeding (Sogawa and Pathak, 1970).

Studies on preference of nymphs and adults for selected rice varieties at IRRI, Philippines showed that non-preference of varieties appeared to be a gustatory response. There were distinct differences in the insect settling behaviour on different rice varieties and strong non-preference for feeding was noticed for resistant varieties (Sogawa and Pathak, 1970). Preference studies with fifth instar and adult BPH indicated strong non-preference to all resistant varieties in Japan (Kaneda and Kisimoto, 1979). Pongprasert and Weerapat (1979) also noticed distinct differences in the settling behaviour of insect on different rice varieties and strong non-preference for settling on resistant varieties.

Vaidya and Kalode (1979) revealed the nature of resistance in selected rice varieties. Observations were taken at an interval of 24, 48 and 72 hours after release of the WBPH, indicated a clear tendency of test insect to move away from the resistant plants after sometime. After 72 hours, there was a reduction in the nymphal population on all the resistant varieties, while there was an increase in the case of susceptible varieties. The numbers of nymphs were 2 to 7 times more on TN 1 than on other varieties. A greater preference of *S. furcifera* nymphs for settling on susceptible varieties than on resistant ones was reported by Khan and Saxena (1985), Lal (1988), and Rath (1995).

Vaidya and Kalode (1981) investigated that the nymphs of WBPH were able to move away from resistant varieties within 2 hours after caging. A similar study has also been observed by Gunathilagaraj and Chelliah (1985), where, the WBPH nymphs preferred susceptible TN 1 more for alightment than on the resistant one. Such a phenomenon has already been visualized earlier (IRRI, 1977; Pablo, 1977; Khan and Saxena, 1985; Lal, 1988 and Kim- Myeongki *et al.*, 1998).

Senguttuvan *et al.* (1991) conducted experiments in laboratory for adult preference and reported that for settling and oviposition, the resistant and moderately resistant varieties were less preferred by the BPH adults than the susceptible varieties. Velusamy *et al.* (1995) also reported that wild rice species (*Oryza officinalis*, *O. punctata*, and *O. latifolia*) which maintained high level of resistance to BPH nymphs, after 48 hours of exposure settled poorly on them as compared to cultivated rice varieties. Wild races were not preferred and significantly more individuals settled on susceptible TN 1 followed by cultivated races.

Nanda *et al.* (1999) witnessed more egg laying by BPH on susceptible rice varieties than resistant ones and inferred that more egg laying on susceptible varieties might have resulted probably due to the favourable chemical environment at the site of oviposition. They also reported that more number of feeding probes on resistant accessions might be because, the insect did not find suitable nutritional substrate more around the probing site and making numerous probes in search of the preferred site of feeding. They also studied the antixenotic modalities of resistance in different age seedlings of rice to both macropterous and brachypterous forms of BPH. The results showed that more macropterous adults settled on susceptible TN 1 (30.4%) compared with 6.2 to 9.0 per cent adults settling on other resistant varieties after 48 h of exposure. With regard to brachypterous adults, susceptible TN 1 harboured a greater number (36.4%), whereas, the resistant accessions could attract 2.5 to 12.4 per cent of the population. The gravid adult females laid maximum number of eggs on TN 1 (57.5 eggs/plant) compared to the resistant varieties and a minimum of 10.5 eggs on ARC 6650 followed by Ptb 33 with 11.5 eggs.

Alagar and Suresh (2007) observed that the settling response of BPH nymphs was more apparent at 24 hours after infestation on all the tested genotypes. The average number of nymphs was lowest on KAU1661 (4.3 per plant) than susceptible control TN1 (7.7 per plant), while on Basmati 370, the highest per cent of unhatched eggs (25.4%) and the lowest number of total eggs laid (130.8 per plant) were recorded.

Singh *et al.* (2008) described that antixenosis was the mechanism of resistance in resistant mutant D 518 than on the susceptible mutant D 1131, susceptible check TN 1 and wild type IR 64, as the female plant hoppers avoided settling and laid fewer eggs both under free choice and no-choice conditions. Yong *et al.* (2012) stated that the number of BPH settled were significantly less on rice lines Q 660 at 48 hours and Q327 plant at 120 hours after release, speculating that antixenotic factors were present in both rice lines.

Shu *et al.* (2009) investigated the effects of zinc on larval reproduction of phytophagous insect *Spodoptera litura* Fabricius in artificial diets, both at ecological and molecular levels. They observed a significantly shorter period of oviposition by *S. litura* females exposed to 300 to 750 mg Zn/kg. The oviposition rate, fecundity and hatchability of female adults treated with 750 mg Zn/kg were significantly lower than

the control. They also inferred that excess zinc made expression of vitellogenin gene downregulated and caused poor accumulation of egg yolk, which ultimately resulted in less number of eggs to hatch.

In a pot culture experiment, antixenosis study showed that the number of BPH nymphs settling on the susceptible genotype was higher as compared to resistant rice accession (He *et al.*, 2013; Qiu *et al.*, 2014).

The pot culture study conducted by Ramesh *et al.* (2014) on the assessment of antixenosis mechanism operating in rice against WBPH indicated that higher numbers of WBPH nymphs were settled on susceptible genotypes compared to resistant ones.

Nine genotypes were evaluated under greenhouse conditions for antixenosis study against BPH. The proportion of insects settled on a test genotype in relation to the susceptible control TN1 was recorded, with significantly lower proportion of nymphs (55.22% - 59.18%), adult males (60.33% - 60.75%), and adult females (80.56% - 79.26%) settled on RP2068-18-3-5 and Ptb33 in relation to those on TN1. Based on number of feeding sites, the test genotypes were ranked in order from the highest to the lowest as RP2068-18-3-5, Ptb33, MR1523, Rathu Heenati, Sinnasivappu, ARC10550, MO1, INRC3021 and TN1. The order was exactly reverse in terms of fecundity expressed as number of eggs laid per female (Sarao and Bentur, 2016).

#### **2.4.2 Antibiosis mechanism**

In the year 1981, Tingey had suggested that antibiosis mechanism in resistant varieties was the reason for pest reduction in a cumulative manner by reduced rate of reproduction. Antibiosis was observed in terms of low population levels by Reddy and Kalode (1981) and similar reduced feeding and slower growth rates of BPH was observed on resistant varieties by Velusamy (1982).

Rodriguez-Rivera (1972) suggested that the nymphs of *S. furcifera* suffered from higher mortality when fed on resistant variety as compared to susceptible TN 1. The adult longevity and life span of WBPH was also adversely influenced by resistant varieties (IRRI, 1972). In a laboratory study, the nymphal development period of *S. furcifera* was observed to fluctuate from 14.7 to 16.0, 11.6 and 10.0 to 10.6 days, respectively on resistant, moderately resistant and susceptible varieties (Choi *et al.*, 1973). They also noticed that there were significant differences in the nymphal period.

The rate of adult emergence of WBPH also differed depending upon the degree of resistance of the rice cultivars. Nymphal period was short (about 10 days) in the susceptible varieties and long (about 14-16 days) in the resistant varieties. They postulated that the lower rate of adult emergence on the resistant varieties may be due to the higher nymphal mortality of the insect in the resistant than on the susceptible varieties.

Sogawa and Pathak (1970) concluded that varieties which permitted the least survival of BPH nymphs were truly resistant. Higher nymphal mortality of BPH caused low rate of adult emergence in the resistant varieties (Lee and Park, 1976). Survival rate determines the effect of antibiosis factor of plants on nymphal stage (Heinrichs *et al.*, 1985). Very low nymphal survival and higher nymphal mortality of BPH were observed on resistant varieties (Misra *et al.*, 1986). Alagar and Suresh (2007) reported that the mean nymphal survival of BPH was the lowest on ADT 45 (42%) followed by ASD 7 (43%) and ARC 10550 (44%) among the genotypes tested.

Pablo (1977) reported that the per cent survival of nymphs of *S. furcifera* on resistant varieties at 15 days after infestation ranged from 12 to 55 per cent and TN 1 had 76 per cent survival. He also observed that development period of *S. furcifera* was prolonged by 4 to 6 days on resistant varieties as against the susceptible TN 1 and that the developmental period of females was longer than the males. Kalode *et al.* (1977) reported that the survival of nymphs of *S. furcifera* at 3 days after caging ranged from 50 to 54 per cent on ARC 5955, ARC 11208 and ARC 11321 as compared to 100 per cent on TN 1. When observations were taken on 9<sup>th</sup> day, the survival further decreased and it was only 10 per cent on ARC 5955 as compared to 45 to 64 per cent on resistant varieties and TN 1 had 75 per cent survival.

Sogawa and Pathak (1970) suggested that prolonged nymphal period on resistant accession 'Mudgo' might be due to low quantities of nutrients required by BPH or due to lack of vital nutrients or due to the toxic substances in the plants leading to herbivory. The nymphal development of BPH was prolonged on resistant varieties than on susceptible ones (Pongprasert and Weeraput, 1979). The developmental time of BPH was mainly affected by the increase in the length of nymphal stage (Cheng and Sun, 1992).

Panda (1976) reported on the effectiveness of micronutrients like boron and zinc against yellow stem borer (YSB) having considerable reduction in the pest load due to induced antibiosis effect of micronutrients. The author further observed that application of zinc and boron as foliar spray on rice suppressed the incidence of gall midge on rice.

Lal (1981) observed significantly lower nymphal survival of *S. furcifera* on resistant (24 to 52 %) and moderately resistant (68 to 76 %) varieties as compared to 96 per cent survival on the susceptible TN 1. In another study, the per cent nymphal survival ranged from 18 to 40 and 52 to 62 per cent on resistant and moderately resistant varieties, respectively on 12<sup>th</sup> day and 12 to 28 and 36 to 48 per cent, respectively on 20<sup>th</sup> day after infestation, whereas, TN 1 had 92 per cent on the 12<sup>th</sup> day and 72 per cent on the 20<sup>th</sup> day after infestation (Lal, 1981). He also indicated significant differences in sexes of *S. furcifera* among resistant, moderately resistant and susceptible varieties. The lower adult emergence of *S. furcifera* on resistant varieties was due to the higher mortality of nymphs. Besides, it was too observed that nymphal period of *S. furcifera* to be 3 to 7 days longer on resistant entries than on the susceptible TN 1.

Heinrichs and Rapusas (1983) reported significantly lower survival of nymphs of *S. furcifera* on resistant rice lines viz., ARCA 10239, ADR 52 and IR 2035-117-3 than that of the susceptible TN 1 at 12 days after infestation. He also observed that the development period of nymph was longest on IR 2035-117-3 (16.1 days) and shortest on the susceptible TN 1 (12.5 days). The similar trend of nymphal period on these two varieties was recorded in different studies (IRRI, 1985; Khan and Saxena, 1985 and Rapusas and Heinrichs, 1985).

Heinrichs and Rapusas (1983) measured the levels of resistance (antibiosis) to *S. furcifera* among the rice varieties for various antibiosis parameters like insect feeding rate, oviposition rate, egg hatchability, survival, length of development period and population growth. Noticeable difference in the levels of antibiosis mechanism was evidenced among varieties. The population growth test was the most useful test to assess the antibiosis levels of resistance among genotypes.

Gunathilagaraj and Chelliah (1985) investigated the components of resistance in seven rice varieties to *S. furcifera*. The resistant varieties had an adverse influence on nymphal survival and duration, as well as on the life span and fecundity of adult females. However, resistance in rice did not have an appreciable effect on egg hatching. The adverse effects of resistant varieties had a cumulative effect on the population build-up through several generations.

The adverse effects of resistant varieties on the growth and development of nymphs, adult survival and reproduction, feeding rate and population growth indicated the operation of antibiosis factors. The resistant varieties reduced the nymphal growth as well as adult longevity of *S. furcifera* (Khan and Saxena, 1985 and Lal, 1988). Gunathilagaraj and Chelliah (1991) studied eight accessions of rice to elucidate the mechanism of resistance against WBPH. Significantly fewer eggs were laid on resistant accessions. Nymphal survival and fecundity was also significantly lower on resistant varieties but hatching of eggs was not affected. The cumulative effect of these accessions through several pest generations favoured the production of males and markedly reduced adult longevity.

Mishra and Misra (1991) reported that in preference tests with seven rice varieties of different resistance ratings, cultivars Pundia and Landi sarakanti were least preferred by *S. furcifera* in free choice tests and no-choice tests, respectively. TN 1 was the most preferred one by the delphacid in both types of tests. Lal *et al.* (1992) reported that *S. furcifera* exhibited a longer nymphal period, a higher mortality rate and reduced egg laying on resistant rice cultivars ARC11324, Balamawee, Ptb19 and Ptb 21 than on susceptible cultivars.

Padhee and Mishra (1993) studied the effect of two zinc formulations ( $ZnSO_4$  and Zn- EDTA chelate complex) on rice leaf folder at Varanasi, Uttar Pradesh; India during *kharif* season. The mean number of freshly folded leaves per 10 hills was highest in plots receiving basal application of 25 kg  $ZnSO_4$  per ha. Plot with Zn-EDTA chelate complex had significantly fewer folded leaves than that in untreated plots. The authors in 1995 further observed that application of Zn-EDTA proved superior to the application of  $ZnSO_4$  in reducing the green leaf hopper (GLH) infestation. Basal application of Zn-EDTA (0.5 kg/ha) followed by top dressing (up to 2 kg/ha) at 25 days after transplanting (DAT) and foliar spray (0.1 %) at 25 DAT and twice more at 15

days intervals significantly reduced the incidence of hoppers in comparison to corresponding treatments with ZnSO<sub>4</sub>.

Oudhia *et al.* (1998) observed low gall midge (GM) infestation in treatments having ZnSO<sub>4</sub> as supplement with different levels of NPK combinations as compared to NPK alone in the rice hybrid, Proagro 6201. The treatment having nitrogen through slow release form with ZnSO<sub>4</sub> also resulted in lower infestation of GM. The study indicated the existence of negative relationship between ZnSO<sub>4</sub> and GM infestation.

Rath and Mishra (1998) reported that application of zinc and iron in form of their sulphatic fertilizer (ZnSO<sub>4</sub> and FeSO<sub>4</sub>) @ 0.2 per cent concentration as foliar spray and @ 25kg/ha as basal application and that of Zn EDTA @ 1kg/ha as basal application and @ 0.2 per cent foliar spray caused decline in WBPH population on rice. The population of WBPH was almost reduced to 50 per cent as compared to control (no treatment).

Fang *et al.* (1998) studied antixenosis, antibiosis and tolerance in 19 rice cultivars against *S. furcifera*. The four japonica cultivars (91-17, Bing 90-98, Bing 850 and Bing 93-63) exhibited stronger antixenosis to adults than all other cultivars tested. The fecundity of *S. furcifera* was significantly reduced on Bing 93-63. Xieyou 9308 and the japonica cultivar Chunjiang 06 showed the most distinct antibiosis. Another indica-japonica hybrid rice combination, Xieyou 413, showed some tolerance to *S. furcifera*.

Laboratory experiments conducted to study the antibiosis mechanism of resistance in 10 rice varieties to *N. lugens*, showed more nymphal survival on the susceptible variety TN 1 (control) as compared with other varieties, namely Mudgo, ASD 7, Rathu Heenati, Babawee, ARC 6650, Utri Rajpan, Udaya, Pratap and Ptb 33. Varietal resistance in rice to *N. lugens* was also studied by estimating the feeding rate on the basis of the amount of honeydew excretion. The results showed that out of 10 varieties evaluated, TN 1 was the most susceptible and Ptb 33 was the most resistant one (Nanda *et al.*, 1999).

Pati (2002) experimented on the efficacy of four micronutrients *viz.*, iron, zinc, copper and manganese in the form of their sulphatic fertilizers applied as foliar spray alone and in combination with basal application against BPH, WBPH and GLH of rice under both field and pot culture experiment. It was revealed that zinc was the most

effective treatment for controlling all above insects together. Under caged condition both zinc and iron affected growth and development of BPH and WBPH causing lower nymphal survival, advancing nymphal development period, producing lower female : male ratio, recording lower growth index and supporting lowest population build-up, which might be ascribed as the induced antibiosis effect of the micronutrient in rice.

Rath (2004) studied on influence of certain micronutrients, viz., iron, zinc, copper and manganese in the form of their sulphatic fertilizers applied as foliar spray alone and in combination with basal application against WBPH, *Sogatella furcifera* (Horvath) on rice under caged condition. Among the micronutrients tested, iron and zinc affected the growth and development of WBPH causing lower nymphal survival, prolonged nymphal duration, lower female : male ratio, lower growth index and population build up. The micronutrients exercised a possible induced antibiosis in rice against WBPH.

Reddy *et al.* (2005) investigated that BPH caged with resistant and moderately resistant genotypes had prolonged nymphal development, slow growth and low survival as compared to the insects caged with susceptible check TN 1. The population build up was 2-12 times lower on resistant and moderately resistant genotypes, gained less weight and excreted less amount of honeydew as compared to TN 1. The fecundity was five times less on resistant and moderately resistant genotypes.

Rath (2006) studied on influence of four micronutrients like iron, zinc, copper and manganese in the form of their sulphatic fertilizers on rice plants as foliar spray (applied @ 0.2 % concentration at 35 and 60 DAT) and both as basal (applied @ 25kg/ha at puddling) and foliar spray at the above doses under caged condition against BPH, *Nilaparvata lugens* (Stal), which revealed that various growth parameters viz., nymphal survival, growth index and population build up were markedly reduced and there was increase in nymphal development period. The variation observed in growth and development is attributed to induced antibiosis effect of the micronutrients tested.

Dash *et al.* (2007) revealed that application of ZnSO<sub>4</sub> along with NPK fertilizers enhanced the nitrogen, potassium, zinc and sulphur content in the rice foliage as compared to application of only NPK fertilizers. Zinc and sulphur uptake in foliage was

also increased when NPK dose was increased. The BPH incidence was inversely related with zinc ( $r = -0.2690$ ) and sulphur ( $r = -0.2689$ ) content.

A study was carried out to assess the genotypic response to *N. lugens* attack and the possible use of these genotypes in BPH management. The resistant genotypes Ptb 33, ADT 45 and ASD 7 and the moderately resistant genotypes CO 43 and KAU 1661 recorded the lowest nymphal survival, growth index and population build up compared with the susceptible genotype TN 1 (Alagar *et al.*, 2007). Studies on the feeding behaviour of *N. lugens* on some selected rice genotypes revealed, low honeydew excretion and higher feeding marks which was related to resistance of rice genotypes against BPH (Alagar *et al.*, 2008).

Dash *et al.* (2008) witnessed that the incidence of YSB was increased with the application of increased dose of NPK fertilizers. But supplement of  $ZnSO_4$  with NPK nutrients recorded lower incidence of YSB in rice both at vegetative (dead heart) and heading stage (white ear head).

Dash and Mukherjee (2009) reported that NPK @ 60:30:30 kg/ha supplemented with  $ZnSO_4$  @ 25kg/ha as basal dose harboured significantly lower BPH than application of corresponding NPK dose alone without  $ZnSO_4$ .

Dash and Mishra (2009) concluded that the WBPH population was significantly decreased markedly with basal supplementation of zinc ( $ZnSO_4$ ) to the NPK nutrients than application of the corresponding NPK fertilizers alone.

Dash *et al.* (2011) revealed that the incidence of YSB was more in *kharif* than in summer season. The extent of damage by YSB increased with increase in doses of NPK levels. The nutrient level 60:30:30 NPK kg/ha with  $ZnSO_4$  recorded minimum borer incidence irrespective of genotypes, thus,  $ZnSO_4$  supplementation possibly exhibited some impact in suppressing YSB incidence.

Sarwar (2011) reported that application of zinc sulphate (33 % - Zn) in the vicinity of the roots using broadcast method at three levels (20, 25 and 30 kg/ha) at 30 DAT reduced the incidence of rice stem borers (*Scirpophaga* species) in rice plant by generating an unfavourable environment for pest and inducing resistance through antibiosis or feeding inhibition. The prevalence of rice stem borer was found to be

highest in control treatment and lowest on 30 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (0.78 ± 0.08 and 2.09 ± 0.11; 1.90 ± 0.18 and 2.56 ± 0.05 dead hearts and white heads on both varieties Shadab and Mehak, respectively).

Madhuri *et al.* (2017) reported that individual nutrient or combination of nutrients i.e. NPK produced more pest incidence of YSB on rice, whereas, application of nutrients (NPK) along with combination of micronutrient like zinc reduced the incidence of the stem borer by 58 per cent during summer and 39.5 per cent during *kharif*, 2015.

### **2.4.3 Tolerance mechanism**

Tolerance refers to a mechanism of resistance in which the host plant exhibits an ability to grow and reproduce normally amidst high pest load or to repair injury to a marked degree as compared to a susceptible host (Painter, 1951).

Paguaia *et al.* (1980) have opined that tolerance mechanism to BPH in resistant rice cultures was noticed, which might be due to low feeding activity compared to the susceptible IR 20. Similarly, Ho (1981) reported that the cultivar Triveni possesses tolerance to *N. lugens* attack both during vegetative and mature growth stages. Yield reduction caused by feeding of 400 numbers of *N. lugens* was about 40 per cent on 35, 50 or 75 days old plants, whereas, it was almost 100 per cent on the susceptible cultivar TN 1.

Lal (1988) found good tolerance of IET 6288, CR 333-6-1, CR 333-6-2 and RP 1800-10-5-8-2 against *S. furcifera*, taking plant damage and plant weight loss into consideration.

The mean functional plant loss index was lowest in ARC 6650 (19.5%) followed by KAU 1661 (22.11%) and ARC 6650 (22.4%) compared to TN 1 (85.4%). The mean tolerance index was low in ASD 7 and Ptb 33 (0.26). ARC 6650 suffered from the lowest dry weight loss of 8.2 mg per mg of insect dry weight produced compared to 90.7 mg in TN 1 (Alagar and Suresh, 2006).

Alagar and Suresh (2007) reported that 30 and 60 day old plants of rice varieties, ARC 10550, KAU1661 and ARC 6650 took significantly longer period (27 to

31 days) for wilting compared to TN 1 (18.2 days) due to low population build up. In spite of supporting higher population of first generation nymphs, KAU 1661 took longer time for wilting, which showed the BPH withstanding capacity of the variety.

Jhansi Lakshmi *et al.* (2012) reported that the wild rice accessions *viz.*, IRGC86476, TRG51, TRP56, IRGC105710, TRP69, TRP36-1, TRP64-2, TRP38-2, TRP70, TRP80434, TRP38, TRP37 and TRP62-1 survived for more than 34 days after exposure to BPH nymphs as compared to 5-6 days in susceptible check TN 1 denoting the presence of high level of tolerance mechanism.

Sarao and Bentur (2016) experimented on nine genotypes of rice under greenhouse conditions for tolerance against BPH. Different parameters *viz.*, days to wilt, functional plant loss index and plant dry weight loss to BPH dry weight produced were recorded and they stated that RP2068-18-3-5, Rathu Heenati and Ptb33 performed better than the susceptible control (TN 1).

## **2.5 Role of zinc on biochemical constituent**

The host nutrition, physiological status of the host plant as well as quantitative variation in the nutritional components can induce the resistance or susceptibility to insects. Sogawa and Pathak (1970) reported that a smaller quantity of amino acids, particularly of asparagine was responsible for resistance to *N. lugens* in the rice variety Mudgo. Pablo (1977) correlated resistance of *S. furcifera* with the nutritive value of host plant and indicated that the total sugar in all resistant varieties except IR 2035-117-3 was higher than the susceptible TN 1 and resistant varieties contained lower quantity of amino acids than the susceptible TN 1. In the year 1983, Gunathilagaraj found that the WBPH resistant rice varieties ARC 10550 and IET 6123 contained more sugars and less amino nitrogen compared to the susceptible TN 1.

Zinc deficiency severely depressed the production of proteins in meristematic tissues and brought about the accumulation of free amino acids and amides (asparagine, glutamine, and alanine) (Kitagishi and Obata, 1986). Peraiah *et al.* (1982) observed that BPH resistant rice varieties had high content of total proteins as compared to plant tissue of the susceptible ones. In the year 2011, Vanitha *et al.* reported that free amino acid content was more in the susceptible variety (TN 1) compared to resistant varieties of BPH (Basmati 370, ASD16 and Ptb33).

The phenolic content of *S. furcifera* resistant variety 'Colombo' was higher than the susceptible TN 1. However, the role of phenolics in resistance of rice varieties to *S. furcifera* was not ascertained (Pablo, 1977 and Lal, 1988). Peraiah and Roy (1979) reported that the BPH resistant varieties of rice, Shakti and CR.95-952-1 had more phenols than the susceptible variety, Ratna in their shoot apices. Peraiah *et al.* (1982) have also witnessed that BPH resistant rice varieties had high content of phenols (435.562 g) in comparison to susceptible ones (370.390 g/100mg of plant tissue). The presence of more amounts of phenols in the resistant varieties may be harmful to the bionomics of BPH and thus, resistant varieties were less preferred by BPH.

Sujatha *et al.* (1987) reported that the total phenolic content had negative correlation with the BPH build up on rice cultivars, suggesting that higher quantity of total phenols was associated with resistance of rice cultivars to BPH. Dharma Reddy and Misra (1995) reported significant differences in the total phenol percentage among the rice varieties against BPH. They observed lowest phenol content in the variety FH-IO9 (5.33%) and the highest in DR-92 (9.10%). Deepa *et al.* (2016) have also noticed that the amount of total phenol was maximum in the leaf sheath of HKR 47 (10.460 mg/g) compared to the susceptible check TN 1 (3.887 mg /g) and the mean amount of total sugars was lowest in Thogai Samba accession (7.409 mg/ g) and maximum in susceptible check TN 1 (16.578 mg/g).

Rath and Mishra (1998) studied on biochemical basis of resistance to WBPH among 7 selected rice varieties, including Ptb 33 and TN 1 (resistance and susceptible check, respectively), at Varanasi, India. Total chlorophyll, total amino acids, total phenols and total sugars contents in tillers of plants were recorded at 50 and 60 DAT. The mean WBPH population was significantly positively correlated with total chlorophyll, total amino acids and total sugars and significantly negatively correlated with total phenols, at both 50 and 60 DAT. The WBPH resistance factor was therefore attributed to low chlorophyll, low sugar, low amino acid and high phenol content.

The effect of some micronutrient fertilizers on the changes in the biochemical component of rice at maximum captivity of WBPH was studied by Rath *et al.* (1999) who reported that the micronutrients like zinc and iron in terms of sulphatic fertilizers applied either as basal or foliar spray brought significant changes in biochemical components like total sugar, amino acid, phenol and chlorophyll content. The WBPH

population recorded at 50 and 60 DAT showed significantly positive correlation with total sugar and amino acid, while it exhibited negative correlation with total phenol content.

Prasad *et al.* (2010) reported that enhancement in dose of zinc from 3 kg/ ha to 7 kg/ha increased the total quantity of phenols from 6.9 mg/100g to 7.9 mg/100g, respectively. Punithavalli *et al.* (2013) explored the insect-feeding induced damage on rice plants and its subsequent effects on the plant biochemical changes in quantitative form. The levels of biochemical such as phenol, ortho-dihydroxy phenol and tannin contents increased in most of the infested resistant rice genotypes *viz.*, Ptb 33, TKM6, LFR831311, *O. rhizomatis* and *O. minuta*, compared to susceptible genotypes *viz.*, TN 1 and IR 36.

Derakhshani *et al.* (2011) observed that in costmary plant, protein and total phenol content, antioxidant capacity, and chlorophyll index were enhanced by zinc supplement action. While the proline and soluble sugars content showed a decreasing pattern with zinc application.

Kumar *et al.* (2013) studied the qualitative and quantitative changes in biochemical constituents in the rice plant. The plant biochemical constituents such as free amino acids, sucrose were lower and phenols and C/N ratio was higher in resistant Ptb 33 compared to susceptible TN1. The high feeding rate and population build up on the susceptible variety TN 1 was positively correlated with higher quantities of sucrose, free amino acids and lower quantities of phenols and low C/N ratio.

Ashrith *et al.* (2017) experimented on biochemical basis of resistance due to infestation by BPH and WBPH in rice and revealed that total sugar content showed significant positive relationship with plant hoppers, whereas, free phenols and soluble protein were negatively correlated.

## **2.6 Effect of zinc on enzyme activity**

Wadhwa *et al.* (2014) investigated that  $Zn^{2+}$  @ 20 mg kg<sup>-1</sup> as soil treatment in cluster bean induced high activity of antioxidative enzymes namely peroxidase (POD) and phenylalanine ammonia lyase (PAL). It might be due to the fact that zinc acts as a cofactor for PAL. Chen *et al.* (2009) have suggested that plant synthesize salicylic acid

(SA) from cinnamate through phenylpropanoid pathway by the activity of PAL (key regulator of the phenylpropanoid pathway). SA induces greater defense against piercing and sucking type of insect pests than the chewing ones (Zhao *et al.*, 2009). SA signaling molecule is involved in local defense as well as induction of systemic resistance. Production of reactive oxygen species (ROS) by SA pathway has been proposed to induce resistance in plants against insect pests. H<sub>2</sub>O<sub>2</sub> induced by SA in plants defends them against various insect pests since, H<sub>2</sub>O<sub>2</sub> actively damages the digestive system of insects leading to reduced growth and development (Maffei *et al.*, 2007). H<sub>2</sub>O<sub>2</sub> acts through signal transduction pathways, which leads to the expression of defence genes (Idrees *et al.*, 2011). Accumulation of H<sub>2</sub>O<sub>2</sub> results in a series of events that stimulates physiological and molecular responses in plants to defend plants against various biotic stresses (War *et al.*, 2011).

PAL also catalyses the biosynthesis of phenolics which have an important role in insect resistance mechanism (Punithavalli *et al.*, 2013). Southerton (1990) also observed direct correlation of induction of PAL activity with phenol content. Oxidation of phenols catalysed by POD is a potential defense mechanism in plants against herbivorous insects and Wadhwa *et al.* (2014) stated that application of zinc increased the POD content. Quinones formed by oxidation of phenols bind covalently to leaf proteins, and inhibit the protein digestion in herbivores and in addition, quinones also exhibit direct toxicity to insects (Bhonwong *et al.*, 2009).

ROS include partially reduced forms of oxygen such as superoxide (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radicals (HO<sup>·</sup>). H<sub>2</sub>O<sub>2</sub> is relatively stable and easily detoxified by catalase and peroxidase enzymes (Grant and Loake, 2000), but to convert O<sub>2</sub><sup>-</sup> (harmful) to O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>, SOD is required and thus, it acts as the first line of enzymatic defense against ROS within a cell (Kumar *et al.*, 2014). In Super oxide dismutase, zinc is attached to copper (Cu/Zn SOD), it seems that zinc has catalytic and copper has building role. Mathpal *et al.* (2015) reported that application of zinc increased SOD activity of two contrasting rice genotypes *viz.*, PD16 (zinc efficient) and NDR359 (zinc inefficient). SOD activity was also observed to decrease in zinc deficiency conditions (Cakmak *et al.*, 1997) and is associated with increase in free radicals oxygen (super oxide) and these free radicals are the toxic substance that has a harmful effect on plant tissue due to lipid peroxidation of membrane and its increased permeability (Marschner, 1995).

Higher accumulation of defense enzymes such as peroxidase and polyphenol oxidase in response to BPH infestation was recorded one day after infestation, and more chitinase activity and pathogenesis-related protein was noticed three days after infestation in resistant and moderately resistant genotypes. The activity was sustained for more than a week after infestation as compared with the susceptible genotype TN 1 (Alagar *et al.*, 2007).

Punithavalli *et al.* (2013) observed greater activities of antioxidative enzymes such as POD and PAL, after rice leaf folder infestation in resistant genotypes than susceptible genotypes.

## **2.7 Role of zinc on nutrient content**

Akshaya (2011) indicated that the highest potassium content was found in the resistant genotype Ptb 33 (2.41 %) and the lowest was in TN 1 (1.13 %). Vanitha *et al.* (2011) have also revealed that among the four rice varieties *viz.*, TN 1, Basmati 370, ASD 16 and Ptb 33, total potassium content was found significantly higher in resistant genotype Ptb 33 as compared to other. Thus, higher potassium content had contributed resistance towards BPH damage. Samiayyan and Janarthan (1988) suggested that the reduction in populations of GLH, WBPH and BPH at high dose of potassium was partly due to the fertilizer enhancing protein synthesis in the sap, making the plant less favourable for the reproduction of sucking pests.

Akshaya (2011) reported that there was a significant difference in zinc content between resistant and susceptible genotypes as zinc plays an important role in many biochemical reactions within the plants and it is also a part of several other enzymes such as SOD and catalase, which prevents oxidative stress in cells. The highest zinc content was observed in HKR 06-47 (84.62 ppm) and the lowest was in TN 1 (42.78 ppm). Similar results were obtained by Cayton *et al.* (1985) who reported significant difference in the content of zinc in the resistant (IR 36) and susceptible variety (IR 26) of rice.

Srivastava *et al.* (2016) witnessed that foliar application of zinc increased the zinc and potash concentration in flag leaves and straw of rice significantly. Due to the fact that zinc fertilizer enhanced the utilization of potassium content of soil and ultimately resulting in higher uptake of potash.

## 2.8 Role of zinc on protein profiling

Sinha *et al.* (2005) have reported that though, there was an overall decline in soluble leaf protein due to leaf folder infestation in rice, the protein profiling showed an increased level of 97 and 38 kDa proteins in all the infested resistant parent and hence, he considered 38 kDa protein may be the defense related protein. Earlier, increase in expression of defense related protein after infestation (Edwards and Wratten, 1983) and induction of a specific protein (53 kDa), due to leaf folder infestation in resistant and moderately resistant rice varieties have been reported by Das *et al.* (1999).

Punithavalli *et al.* (2013) studied on sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE) analysis for total protein profiling of healthy and leaf folder infested genotypes of rice and revealed the enhanced expression of a high molecular weight (> 97 kDa) protein in all the genotypes. Besides, there was also an increased induction of a 38 kDa protein in leaf folder infested resistant genotypes, which was absent in uninfested plants and this may be considered as a defense related protein. He concluded that there was an increase in expression of a specific protein due to leaf folder infestation, which acted as a key for identification of leaf folder tolerant or resistant genotypes.

## MATERIALS AND METHODS

The present investigation on the role of zinc in inducing resistance in rice to the white backed plant hopper (WBPH) comprised various field, pot culture and laboratory experiments. All the field experiments were carried out at the Central Research Farm, Department of Entomology, College of Agriculture, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar. The pot culture experiments were also conducted in the Department of Entomology, College of Agriculture, OUAT. Various biochemical analysis of plant samples were undertaken in different laboratories of Department of Entomology, Soil Science and Agricultural Chemistry and Agricultural Biotechnology, College of Agriculture, OUAT. The materials used and the methods employed are discussed hereunder in detail.

### 3.1 Location, climate and soil

The experimental plot was located in a medium land of Central Research Farm, OUAT, Bhubaneswar. The area comes under the Coastal Plain Agroclimatic Zone of Odisha. Bhubaneswar is located at latitude of 20° 15' N and longitude of 85° 52' E, with an altitude of 25.9 m above the mean sea level and 64 km away from Bay of Bengal, towards west direction. The climate of Bhubaneswar is sub-tropical and the city experiences an average annual rainfall of 1500 mm. Monsoon generally coincides with the second week of July in the *kharif* season with its peak during August-September. The maximum temperature of 34-40° C remains for a period of 2 months in May and June and from 13-15° C during December-January. However, high temperature of around 42° C persists in the month of May. The relative humidity ranges from 57.5 to 91.5 per cent. The detailed meteorological data of the locality during the experimental period is presented in Appendix-1.

The soil type of the experimental plot was sandy loam and its characteristics with nutrient status as estimated in the laboratory of Department of Soil Science and Agricultural Chemistry, College of Agriculture, OUAT are briefly mentioned in Table 1.

**Table 1. Information on the soil characteristics of the experimental plot**

<b>Characteristics</b>	<b>Result</b>
Texture	Sandy loam
Available Nitrogen	180.00 kg/ha
Available Phosphorous	17.20 kg/ha
Available Potassium	167.60 kg/ha
Zinc	0.467 mg/kg
Electrical conductivity	0.107 ds/m
pH	6.0

### **3.2 Field Experiment**

The field experiment was conducted for three season's viz., *kharif*, 2016, summer, 2016-17 and *kharif*, 2017. The detail plan of work is described hereunder.

#### **3.2.1 Variety under study**

Taichung Native 1 (TN 1), a susceptible medium duration rice variety (135 days) was taken under study as the test variety in all the seasons.

#### **3.2.2 Nursery raising**

The nursery bed was brought to fine tilth and bunds were made around the nursery plots. Seeds of rice variety TN 1 were sown in lines on the well prepared nursery bed during 2<sup>nd</sup> week of July for *kharif*, 2016 and *kharif*, 2017 and 2<sup>nd</sup> week of December for summer, 2016-17. Fertilizers were applied in accordance with recommendations to ensure healthy seedlings and the nursery bed was irrigated as per the need.

#### **3.2.3 Preparation of main field and transplanting**

The main experimental plot was thoroughly ploughed by tractor to get the fine tilth and all weeds and stubbles were removed from the field. The field was inundated with water, puddled twice and then levelled.

Twenty one days old seedlings were transplanted at a spacing of 15 cm × 10 cm, with two seedlings per hill. Gap filling was done after a week to ensure uniform plant stand in each plot. Other field operations like irrigation/drainage as well as weeding were taken up on need basis, following agronomic recommendation. All the basal

treatments along with recommended dose of fertilizer 80 : 40 : 40 Kg NPK/ha was applied prior to transplanting.

### 3.2.4 Irrigation and fertilizer application

The experimental field was irrigated on need basis until the establishment of seedlings after transplanting. The field was irrigated frequently thereafter to maintain 1cm standing water uniformly and the irrigation was completely stopped 10 days prior to harvesting of crop.

Nitrogen (N), Phosphorous ( $P_2O_5$ ) and Potash ( $K_2O$ ) were applied in the form of Urea, Di ammonium phosphate and Muriate of potash, respectively, at the recommended dose of 80 : 40 : 40 Kg/ha. One third of N and the entire amounts of  $P_2O_5$  and  $K_2O$  were applied as basal dose after puddling. The remaining N was applied in two spilt doses; one at peak tillering stage and the other at panicle initiation stage. The fertilizers were applied by broadcasting uniformly in the plot.

### 3.2.5 Details of treatments

Two formulations of zinc i.e. zinc sulphate ( $ZnSO_4 \cdot 7H_2O$  - 21% Zn) and chelated zinc (Zn EDTA-12% Zn) were taken with different combinations along with an untreated check. Various treatments imposed over the seasons are presented in Table 2.

**Table 2. Details of the treatments imposed over seasons**

Treatment no.	Treatment	Method of application	Dose	Source
T <sub>1</sub>	ZnSO <sub>4</sub>	Basal	25kg/ha	Good Earth, IRC Village, Nayapalli, Bhubaneswar
T <sub>2</sub>	Zn EDTA	Basal	40 kg/ha	-do-
T <sub>3</sub>	ZnSO <sub>4</sub>	Foliar spray twice at 30 and 45 DAT	0.5% (5g/l of water)	-
T <sub>4</sub>	Zn EDTA	Foliar spray twice at 30 and 45 DAT	0.8% (8g/l of water)	-
T <sub>5</sub>	T <sub>1</sub> + T <sub>3</sub>	-	-	-
T <sub>6</sub>	T <sub>2</sub> + T <sub>4</sub>	-	-	-
T <sub>7</sub>	T <sub>1</sub> + T <sub>4</sub>	-	-	-
T <sub>8</sub>	T <sub>2</sub> + T <sub>3</sub>	-	-	-
T <sub>9</sub>	Control	-	-	-

### 3.2.6 Design and layout of experimental plot

The experiment was laid out in a randomised block design (RBD). There were a total of nine treatments consisting of different zinc formulations along with an untreated check. The experiment was replicated thrice and each subplot measured (3 m × 3.5 m). Each subplot replication wise was separated from others by bunds. Drainage facility as well as irrigation channels were provided to the experimental plots for necessary irrigation and drainage. The layout of the experiment is presented in Figure 1 and the field experimental plot is presented in Figure 2 to 4.

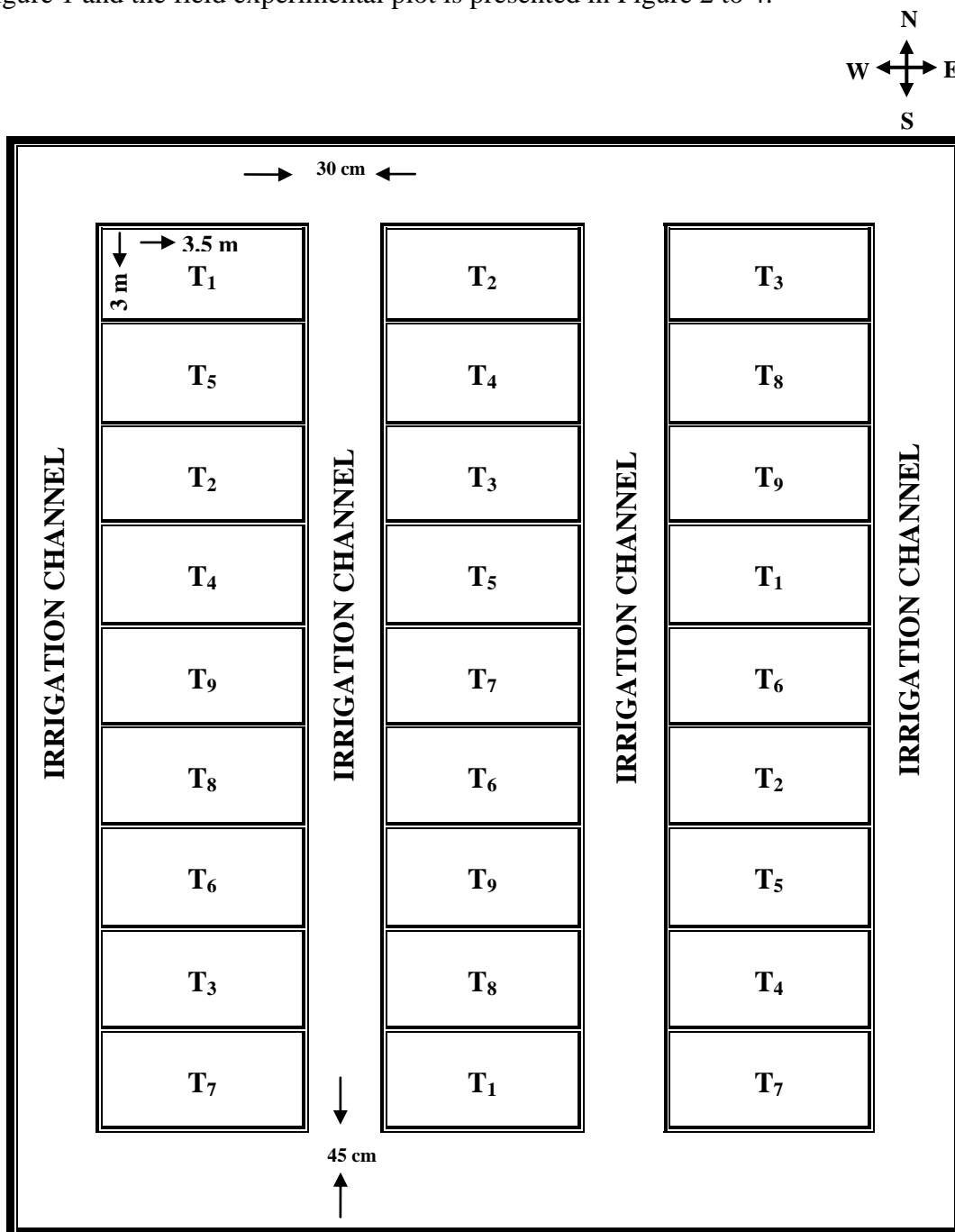


Fig. 1: Layout plan of the field experimental plot (for all seasons)



**Fig. 2: A view of field experiment during *kharif*, 2016**



**Fig. 3: A view of field experiment during summer, 2016-17**



**Fig. 4: A view of field experiment during *kharif*, 2017**

### 3.2.7 Observations

Observation on WBPH population per hill were recorded on randomly selected ten hills in each plot at ten days interval starting from 40 days after transplanting (DAT) up to 80 DAT. The visual counting was made during morning hour when hopper activity was minimum. Various plant growth parameters *viz.*, plant height, total number of tillers per hill and yield attributing traits *viz.*, number of panicles per square meter, number of grains per panicle were also taken at each observation day to find whether there exist any relationship between the WBPH population, individual plant growth parameter and yield attributing trait or not.

Data on grain yield with respect to each treatment was recorded separately and converted to q/ha.

### 3.3 Pot culture experiments

#### 3.3.1 Raising and maintenance of seedlings

The soil mixture for filling of the pots was prepared by mixing the soil thoroughly with farm yard manure. The mixture was analysed for physico-chemical properties *viz.* texture, pH, electrical conductivity, zinc content, available nitrogen, phosphorous and potash content (Table 3) before filling of the pots. Healthy seeds of the test variety TN 1 were sown at different time intervals in earthen pots containing puddled soil to get the seedlings of various ages. Twenty days after sowing, the rice seedlings were pulled out and transplanted in 10kg capacity pots filled with fertilizer enriched (100:50:50 kg NPK /ha) puddled soil, @ 2 seedlings/ pot for conducting various experiments. The potted plants were maintained healthy by applying irrigation and necessary fertilizers, on need basis. Seeds of TN 1 were sown periodically in the pots to ensure regular supply of rice plants for mass rearing of the WBPH.

**Table 3. Physico-chemical properties of the soil mixture tested in the laboratory**

Sl. No.	Parameters	Result
1	Texture	Sandy loam
2	Available Nitrogen	160.50 kg/ha
3	Available Phosphorous	18.60 kg/ha
4	Available Potassium	159.00 kg/ha
5	Zinc	0.481 mg/kg
6	Electrical conductivity	0.105 ds/m
7	pH	6.1

### **3.3.2 Mass rearing of WBPH**

The initial collection of WBPH adults was made from the Central Research Station, OUAT, Bhubaneswar as well as from rice field of some local farmers. These adults were released on previously grown one month old plants of rice variety TN 1 sown in earthen pots. The plants were covered by Mylar cages of 45 cm height with open top end being covered by muslin cloth. The hopper adults were released in the cages using an aspirator for multiplication. The old plants were replaced periodically with fresh potted plants of the same age. Separate pots containing various age groups of TN 1 plants were maintained throughout the experimental period to procure sufficient number of the test insects. Separate cages were maintained for oviposition and rearing of hopper. For obtaining newly emerged adults, all the existing adults of one rearing cage were removed from the cage. The adults that emerged by the next day, were taken as the newly emerged ones. For newly hatched nymphs, healthy potted rice plant of variety TN 1 (about one month old) was exposed to a large number of adults for oviposition under separate cages. After 24 hours, the pots were replaced with fresh ones. The oviposited plants were covered with cylindrical Mylar cages until hatching of nymphs. Care was taken to keep the rearing cage away from its predator (Figure 5).

### **3.3.3 Study of mechanisms of resistance**

To understand the mechanisms of resistance *viz.*, antixenosis, antibiosis and tolerance mechanisms through application of zinc, various studies and experiments were conducted which are described below in pertinent sub headings.

Twenty one days old seedlings of TN 1 rice variety were transplanted on 10 kg capacity pots filled with fertilizer enriched puddled soil and calculated amount of treatment chemicals, where basal applications were required. After application of recommended fertilizer dose and basal treatment in respective pots, they were covered by the Mylar cages of 45 cm height with top end covered by fine muslin cloth. The plants were maintained as such under cage. At 30 and 45 DAT, foliar treatments were imposed on the plants. From 46 DAT the test insects were released into the cages as per the study requirement.



**Fig. 5 (a)**



**Fig. 5 (b)**



**Fig. 5 (c)**



**Fig. 5 (d)**

**Fig. 5: Mass rearing of WBPH in rice**

### **3.3.3.1 Antixenosis mechanism induced by zinc**

#### **3.3.3.1.1 Nymphal alightment**

At 46 DAT, nine numbers of pots representing nine treatments were arranged in a circular fashion and simultaneously confined under a mosquito net, as one experimental unit. Each unit was considered as a replication. Three such replications were run in the present study (Figure 6). Around 300 numbers of second and third instar nymphs collected in specimen tube with the help of aspirator from the rearing cages were released separately into each experimental unit. The total number of nymphs settling on each treated plants were counted at 6, 24, 48 and 72 hours after release. After each observation, the seedlings were disturbed to facilitate fresh orientation. The data on nymphal orientation and settling at different hours of observation were recorded and expressed as per cent alightment (Heinrichs *et al.*, 1985).

#### **3.3.3.1.2 Adult alightment**

In this experiment the exact set up was placed as described in the nymphal alignment study. It was replicated for three times (Figure 7). Nearly 100 adults collected from the insect rearing house were released on the central pot. Observation on adult settlement on different treatments was recorded at 6, 24, 48 and 72 hours after release. The data on adult orientation and settling at different hours of observation were recorded and expressed in per cent adult alightment (Heinrichs *et al.*, 1985).

#### **3.3.3.1.3 Oviposition study**

In another set of experimental pots, one gravid female was released into each pot at 46 DAT and the experiment was replicated five times (Figure 8). After 7 days of release, the surviving females were removed from the pot. The potted plants were continuously observed to note the emergence of nymphs. The number of emerging nymphs was noted from each pot every day and transferred to rearing cage. This observation was continued till no nymphal emergence was observed. Then the plants were dissected and observed under a binocular research microscope attached to computer and searched for number of unhatched eggs. Thus, with respect to each treatment, the number of nymphs emerged from a pot and the number of unhatched eggs found from that pot accounted for the total number of eggs laid by the female.



**Fig. 6: Pot culture experiment on nymphal alightment**



**Fig. 7: Pot culture experiment on adult alightment**



**Fig. 8: Pot culture experiment on oviposition study**

Thus, the total fecundity and unhatched eggs were recorded, treatment wise. Unhatched eggs were expressed as percentage of total, which is sum of number of nymphs counted and the number of unhatched eggs (Heinrichs *et al.*, 1985).

$$\% \text{ Unhatched eggs} = \frac{\text{Number of unhatched eggs}}{\text{Number of nymphs} + \text{Number of unhatched eggs}} \times 100$$

### **3.3.3.2 Antibiosis mechanism induced by zinc**

#### **3.3.3.2.1 Nymphal survival**

Ten numbers of one day old first instar nymphs collected from rearing cages by means of aspirator were released at 46 DAT into each cage containing treated plants (Figure 9). Each treatment faced five replications. The plants were observed daily and the number of adults were counted whenever they emerged and removed from the plant. The number of nymphs that survived and developed into adults indicated the per cent nymphal survival (Heinrichs *et al.*, 1985). It was calculated by the formula given below.

$$\% \text{ Nymphal survival} = \frac{\text{Number of adults emerged} + \text{number of nymphs survived}}{\text{Total number of nymphs released}} \times 100$$

#### **3.3.3.2.2 Nymphal development**

The set up for nymphal duration study was the same as above. Ten number of 1<sup>st</sup> instar nymphs of WBPH were released into each cage and the nymphs were observed daily for ecdysis (Figure 9). The number of days to reach the adult stage was recorded in all the five replications (Pongprasert and Weeraput, 1979).

#### **3.3.3.2.3 Sex ratio**

The adults that were obtained from nymphal development study were observed for male and female sexes separately. Sex ratio in form of numerical of female : male ratio was computed with respect to each treatment in all the five replications.

#### **3.3.3.2.4 Growth index**

Growth index was calculated by the help of data obtained from nymphal development and nymphal survival studies. The formula used to calculate growth index (Alagar and Suresh, 2007) was followed.

$$\text{Growth index} = \frac{\text{Mean nymphal survival}(\%)}{\text{Mean nymphal period (days)}}$$

### **3.3.3.2.5 Adult longevity**

At 46 DAT, ten pairs of freshly emerged male and female adults were released into each cage containing treated plants. There were five replications per treatment (Figure 10). Both sexes of adults were observed daily for their longevity (Rodriguez – Rivera, 1972).

### **3.3.3.2.6 Population build up**

In this study, one adult gravid female insect of 3-4 days old was released into each cage with necessary treatment at 46 DAT. The experiment was replicated five times (Figure 11). After one month of release, the population of WBPH developed was counted, replication wise (Heinrichs *et al.*, 1985).

### **3.3.3.2.7 Feeding potential**

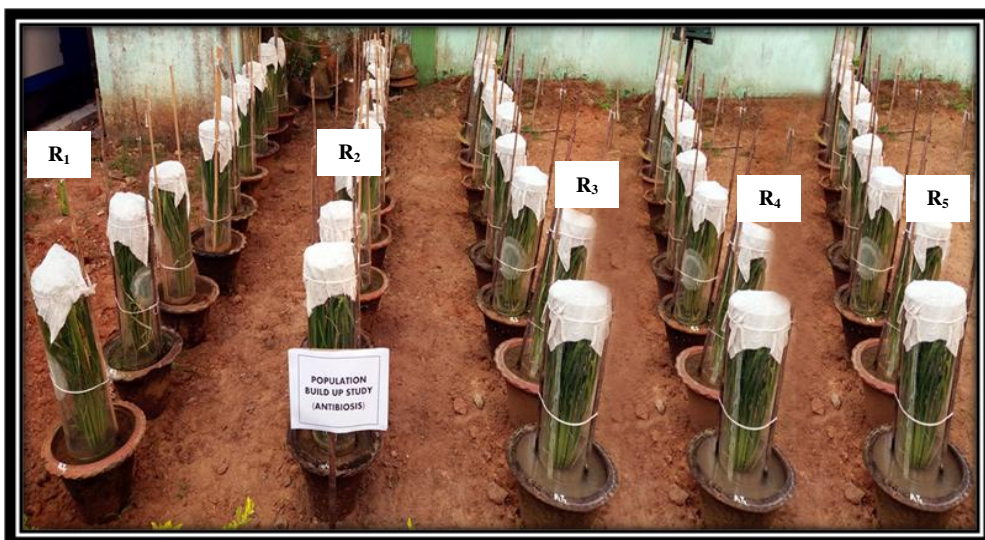
To determine the extent of feeding by WBPH on different treatments, honeydew excretion method as developed by Paguia *et al.* (1980) was followed. The potted plants with necessary basal and foliar treatments were used in this study. At 46 DAT, for each pot, a card board was cut into square shapes of 15 cm × 15 cm size and a hole was made at the centre of the square. The card board was passed through the plant (only one plant was maintained in each cage) and put over the pot. The same way a medially perforated plastic petridish was kept over the card board and the hole was plugged with non-absorbent cotton to prevent moisture absorption. Over to that, a Whatman No. 1 filter paper perforated centrally and cut along one side was placed over the petridish encircling the base of the rice plant. A small plastic cup also centrally perforated at the base was passed through the seedling in an inverted manner over the filter paper. The cup was firmly fixed by cellotape (Figure 12). Five brachypterous adult females previously collected and starved for about five hours but water satiated was introduced gently by the aspirator through the hole of plastic cup and which was then plugged by cotton to prevent the escape of insect. The adults were thus, confined to the basal portion of rice plant because of the plastic cup that constituted the feeding chamber. After 24 hours of release of adults, the chamber was dismantled and the filter paper was taken out and sprayed with 0.001 % ninhydrin in acetone solution. The filter papers were oven dried at 100<sup>0</sup> C for 5 minutes. Purple or violet spots appeared on filter paper because of amino acid contents, which were encircled and traced on a tracing paper and the tracing paper was placed over 1 mm<sup>2</sup> graph paper. After that the area of honey dew in terms of mm<sup>2</sup> was measured and this experiment was replicated five times.



**Fig. 9: WBPH nymphal survival and development on zinc treated rice plant**



**Fig. 10: Pot culture experiment for adult longevity in WBPH**



**Fig. 11: Experiment on population build up of WBPH in rice treated with zinc**



Fig. 12 (a)

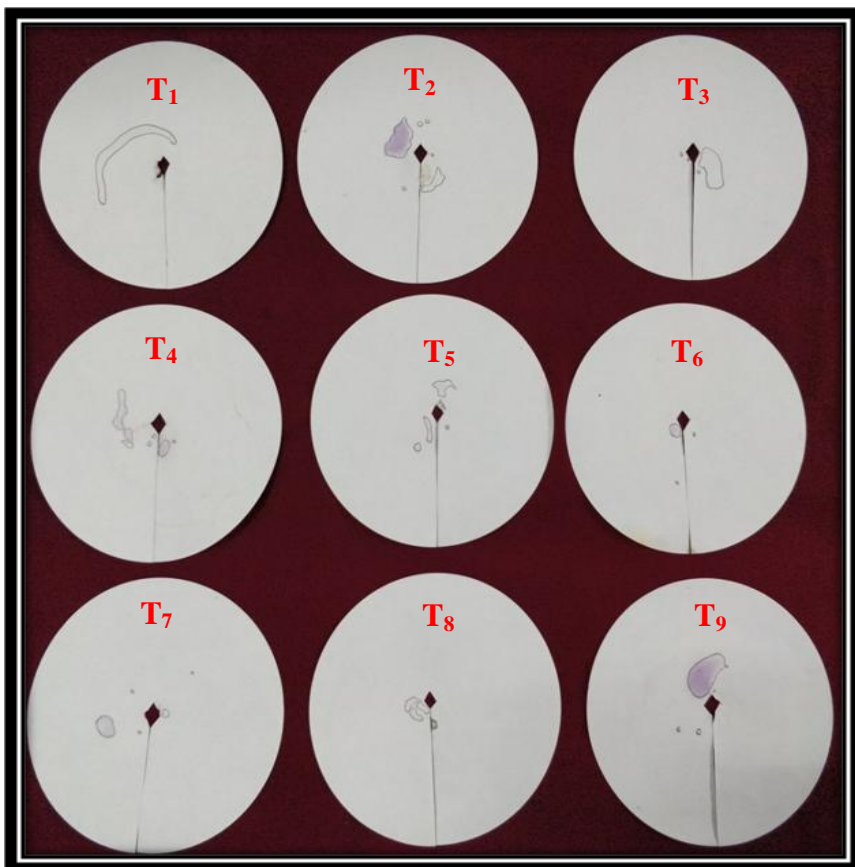


Fig. 12 (b)

Fig. 12: Experiment on feeding potential by honey dew excretion method

### **3.3.3.2.8 Weight gain by adult**

In this study, five pairs of freshly emerged female and male of WBPH were weighed individually in small vials and released on treated plants kept in cages at 46 DAT. The insects were allowed to feed for 48 hours, then collected individually and reweighed to record the difference in weight gain. Each treatment faced three replications (Ramulamma, 2014).

### **3.3.3.3 Tolerance mechanism induced by zinc**

#### **3.3.3.3.1 Days to wilt**

In another set of experiment, 100 numbers of 2<sup>nd</sup> and 3<sup>rd</sup> instar nymphs of WBPH were released on the potted rice plants at 46 DAT, imposed with various treatments. The experiment was conducted with three replications (Figure 13). The period of time taken for wilting of plants after WBPH infestation was taken as a measure of tolerance. The plants were observed daily for plant health and vigour. The time taken by the plants to wilt in different treatments was recorded (Ramulamma, 2014).

#### **3.3.3.3.2 Loss in plant biomass**

In this study, 100 numbers of mixed populations of second and third instar nymphs of WBPH were introduced into treated plants kept in cages at 46 DAT. Similar set of uninfested plants were maintained simultaneously. The population pressure was applied for two weeks only. At harvest, grains of each replication as well as remaining plant parts were oven dried at 60<sup>o</sup>C for 72 hours, after which the weight of the samples of both infested and uninfested plants were recorded separately. Each treatment was replicated three times. The extent of loss in biomass production as a result of nymphal feeding was calculated based on the extent of loss in biomass production of infested and uninfested plant with respect to each treatment (Rath, 1995).

#### **3.3.3.3.3 Functional plant loss index (FPLI)**

To study the level of tolerance, 100 numbers of mixed populations of second and third instar nymphs of WBPH were introduced into each respective caged plant at 46 DAT. Similar set of uninfested plants were maintained simultaneously. The insects were allowed to feed on the plants till the control plant (without any zinc treatment) showed wilting and drying symptoms. At that time, all the plants were removed from



**Fig. 13 (a): Pot culture experiment on study of induced tolerance mechanism in zinc treated rice**



**Fig. 13 (b): Zinc treated plant**



**Fig. 13 (c): Zinc untreated plant**

**Fig. 13: Tolerance mechanism study in zinc treated rice plant**

the pots along with roots, washed thoroughly, air dried for three hours, then dried in an oven at 70°C for 60 hours and weighed. Each treatment was replicated three times. The FPLI was calculated based on the dry weight of infested and uninfested plant (Panda and Heinrichs, 1983).

$$\text{FPLI} = 1 - \left[ \frac{\text{Dry weight of infested plant}}{\text{Dry weight of uninfested plant}} \right] \times 100$$

### **3.4 Analysis of biochemical components of rice plant**

#### **3.4.1 Collection of fresh sample for biochemical analysis**

Leaf samples from the pot culture experiment at 60 DAT (highest population build up under pot culture study) were collected for analysis of various biochemical parameters. The crop age of maximum WBPH incidence under field study more or less coincided with the crop age in pot culture study.

##### **3.4.1.1 Estimation of total chlorophyll content**

The chlorophyll content of the leaf sample was quantified according to the method given by Arnon (1949). About one gram of fresh sample was homogenized in a pre-cooled mortar and pestle using 10 ml of 80% (V/V) acetone. The extract was centrifuged at 10,000 rpm for 10 minutes and the supernatant was transferred to a 100 ml volumetric flask. Pellet was re-homogenised in 80% acetone. The process was repeated until colourless pellet was obtained. The absorbance of the supernatant was read at 645 and 663 nm against the solvent (80% acetone) blank on a spectrophotometer. The amount of chlorophyll present in the extract was calculated using the following equation:

$$\text{Total chlorophyll (mg/g tissue)} = 20.2(A_{645}) + 8.02 (A_{663}) \times V/1000 \times W$$

Where,

A = Absorbance at specific wavelengths

V = Final volume of chlorophyll extract in 80% acetone

W = Fresh weight of the tissue extracted

##### **3.4.1.2 Estimation of total phenol**

Total phenol content of the leaf sample was estimated as per the procedure outlined by Malick and Singh (1980). One gram of fresh sample was ground in 10 ml

of 80 per cent ethanol. The homogenized sample was centrifuged at 10,000 rpm for 20 minutes and the supernatant was collected. The residue was re-extracted and the supernatant was pooled. The supernatant was evaporated to dryness. The residue was dissolved in 5 ml of distilled water. Aliquots of 0.5 and 1.0 ml were pipetted out into test tubes and volume was made up in each tube to 2.0 ml with distilled water. To each test tube 5.0 ml of 20% Na<sub>2</sub>CO<sub>3</sub> was added and the contents were allowed to stand for 10 minutes. One ml of Folin-Ciocalteu reagent was added in all the tubes. After 30 minutes, the absorbance value was measured at 660 nm by using spectrophotometer against blank. A standard curve was prepared using different concentrations of Catechol. With the standard curve, the concentration of total phenol present in the samples was calculated and expressed in mg/g of sample.

#### **3.4.1.3 Estimation of total free amino acids**

Total free amino acids content of the leaf sample was estimated as per the procedure outlined by Moore and Stein (1984). One gram of the sample was ground well with a pestle and mortar in 10 ml of 80 per cent ethanol. The homogenate was centrifuged and the supernatant was used for estimation. sample extract (0.1 ml) was pipetted out in the test tubes. One ml of ninhydrin was added and the volume was made upto two ml in all the tubes. The tubes were kept in the boiling water bath for 20 minutes. Five ml of the diluent solvent (equal volume of water and n – propanol) was added, mixed well and allowed to stand for 15 minutes. The intensity of the purple colour was read at 570 nm. Total free amino acid was determined by using calibration curve prepared by leucine and expressed as mg g<sup>-1</sup> amino acids of plant sample.

#### **3.4.1.4 Estimation of total soluble protein**

The soluble protein in the enzyme extract (refer 3.5.1) was precipitated by 20% trichloro acetic acid, centrifuged and residue dissolved in 0.1N sodium hydroxide (NaOH) solution and was determined by the method of Lowry *et al.* (1951).

Reagent A: 2% sodium carbonate in 0.1N sodium hydroxide

Reagent B: 0.5% copper sulphate in 1% sodium-potassium tartrate

Reagent C: prepared by mixing 50ml of reagent A with 1ml of reagent B

Reagent D: Folin-Ciocalteu reagent diluted with water in 1: 1 ratio

To 1ml extract of the solubilized protein, obtained after precipitation, 5ml of reagent C was added. To this, 0.5ml of reagent D was added with simultaneous mixing. After 30 min, the absorbance was read at 660 nm. The protein content in mg g<sup>-1</sup> fresh weight of the tissue was calculated by calibrating the absorbance with bovine serum albumin as standard.

#### **3.4.1.5 Estimation of total soluble sugar**

Estimation of total soluble sugar content of the leaf sample was done as per the procedure outlined by Mahadevan and Sridhar (1986). One gram of fresh sample was hydrolysed by keeping it in a boiling water bath for 3 hours with 5 ml of 2.5 N HCl. The sample was neutralized using solid sodium carbonate and the volume was made up to 100 ml. The sample was centrifuged and the supernatant was used for analysis. Aliquots of 0.5 and 1.0 ml were taken from the sample and the volume was made up to 1 ml using distilled water in all the test tubes. Four ml of anthrone reagent was added and the test tubes were kept in boiling water bath for eight minutes. Tubes were then cooled immediately and the green colour was read at 630 nm by using spectrophotometer. A standard curve was prepared using different concentrations of glucose. The amount of soluble sugars present in the sample was calculated using the standard graph.

#### **3.4.1.6 Estimation of proline content**

The proline content of the leaf sample was determined by acid ninhydrin method given by Bates *et al.* (1973). Fresh samples (0.5 g) were homogenised by mortar and pestle with 10 ml of 3% aqueous sulphosalicylic acid and filtered through Whatman No. 2 filter paper. About 2 ml of extract was taken in test tube and to it 2 ml each of glacial acetic acid and ninhydrin were added. The reaction mixture was boiled in a water bath at 100<sup>0</sup>C for 1 hour and cooled to room temperature. To the reaction mixture, 4ml of toluene was added. After thorough mixing, the chromophore (Toluene) layer was aspirated and warmed to room temperature. The absorbance of red colour was measured in a spectrophotometer at 520 nm using L-proline as a standard. The amount of proline in the sample was calculated using a standard curve prepared from L- proline and expressed as µg/g fresh weight of the sample.

### **3.5 Enzymatic assay**

#### **3.5.1 Preparation of enzyme extract**

The entire procedure for preparing enzyme extract of leaf sample collected from pot culture experiment at 60 DAT was done at 0-4<sup>0</sup> C. Enzyme extract for superoxide dismutase, peroxidase and phenylalanine ammonia lyase was prepared by freezing the weighed amount of samples (0.2 g) in liquid nitrogen to prevent proteolytic activity followed by grinding with 5 ml extraction buffer (0.1 M phosphate buffer pH 7.5, containing 0.5 mM EDTA) in a previously chilled mortar using glass beads as abrasive. The enzyme extract was centrifuged for 20 min at 15000 rpm in a refrigerated centrifuge at 0-4<sup>0</sup> C and the supernatant was used as enzyme source.

##### **3.5.1.1 Estimation of Superoxide dismutase (SOD) activity**

SOD activity was determined using the method of Dhindsa *et al.* (1981) which is based on formation of blue coloured formazone by nitro-blue tetrazolium chloride (dye) and O<sub>2</sub><sup>-</sup> radical. The reaction mixture (3 ml) consisted of 0.2 ml Methionine (200 mM), 0.1 ml Nitroblue tetrazolium chloride (NBT) (2.25 mM), 0.1 ml EDTA (3.0 mM), 0.1 ml Riboflavin (60 μM), 0.1 ml Sodium carbonate (1.5 M), 1.5 ml Phosphate buffer (100 mM, pH 7.8), 0.8 ml distilled water and 0.1 ml enzyme extract. The reaction mixture without enzyme served as blank. All the test tubes except blank were then exposed to fluorescent light (30 w) for 15 min. The absorbance was then recorded at 560 nm and the enzyme activity was expressed in units min<sup>-1</sup> mg<sup>-1</sup> protein. One unit of activity is the amount of enzyme required to inhibit 50 per cent initial reduction of NBT under light.

##### **3.5.1.2 Estimation of peroxidase (POD) activity**

Peroxidase activity in the extract was assayed according to the method of Castillo *et al.* (1984). The final volume reaction mixture (3 ml) contained 1.0 ml potassium phosphate buffer (100 mM, pH 6.1), 0.5 ml Guaicol, 0.5 ml of 30% H<sub>2</sub>O<sub>2</sub>, 0.1 ml enzyme extract and 0.9 ml distilled water. Increase in the absorbance due to formation of tetra guaicol was recorded at 470 nm and it was expressed in units min<sup>-1</sup> mg<sup>-1</sup> protein. The enzyme activity was calculated using the extinction coefficient of tetraguaiacol (26.6 mM/cm).

### **3.5.1.3 Estimation of phenylalanine ammonia lyase (PAL) activity**

The activity of PAL was measured following the method of Biehn *et al.* (1968). The reaction mixture consisted of 4ml of 14mM phenylalanine (0.231 g phenylalanine dissolved in ethanol and volume made to 100ml with borate buffer, pH 8.8) and 0.5ml of the enzyme extract. After incubation at 37°C for 2 hours, 0.1ml of 6N HCl was added to stop the reaction. The contents were then extracted twice with an equal volume of diethyl ether and evaporated to dryness. The residue was dissolved in 10 ml of 50 mM NaOH (2 g/L) and absorbance was taken at 290 nm. A blank was run simultaneously. Enzyme activity was expressed in units with 1 unit = 1  $\mu$  mole t-cinnamate accumulated  $\text{h}^{-1} \text{mg}^{-1}$  protein.

## **3.6 Analysis of nutrient content of rice plant**

### **3.6.1 Preparation of oven dried sample**

Plants from pot culture experiment at 60 DAT were uprooted and washed with distilled water. These samples were first air dried and then again dried at 60°C in a hot-air oven for 24 hours. After drying it was cut into small pieces and made into powdered form. These powdered samples were sieved through a 100 mesh screen and stored in the sealed containers at 4°C for further use.

### **3.6.2 Di-acid digestion of plant sample**

Plant samples were digested by di-acid mixture i.e.  $\text{HClO}_4 + \text{HNO}_3$  (3:10). Dried plant samples (0.5 g) was pre-digested with 10 ml concentrated  $\text{HNO}_3$  for 6 hrs. Further, it was digested in hot plate adding 10 ml  $\text{HNO}_3$  and 3 ml of  $\text{HClO}_4$  with temperature up to 200°C until the content is reduced to 2-3 ml. After that 10 ml of 2 N HCl was added and content was warmed and filtered through Whatman number 42 filter paper. Volume was made up to 100 ml.

#### **3.6.2.1 Estimation of potassium content**

The di-acid digested samples were used to estimate the potassium content using digital flame photometer (Jackson, 1973). A standard curve was prepared with 0, 25, 50, 100 ppm potassium solutions by adjusting the flame photometer with 100 ppm solution. Fifty ml of di-acid digest was taken and evaporated to dryness on water bath. The residue left was dissolved in water and transferred to 100 ml volumetric flask making up the volume. The solution was positioned to the capillary of the flame photometer and the reading was noted in ppm.

### 3.6.2.2 Estimation of zinc content

The di-acid digested samples were used to estimate the zinc content by atomic absorption spectrophotometer (Lindsey and Norwell, 1978). The blank solution ( $0 \mu\text{g ml}^{-1}$ ) is used to zero the atomic absorption spectrophotometer. A graph of absorbance versus concentration of standard solutions is plotted on a graph paper. After setting the instrument, the samples were aspirated and the absorbance was read. The zinc concentration was found out against absorbance from the curve.

### 3.7 Analysis of protein profile

Gel banding pattern of protein was carried out by Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) method according to Lagrimini and Rothstein (1987). Medium range molecular marker was used for this qualitative study.

Resolving solution was prepared by mixing 30% acrylamide stock (10 ml), resolving gel buffer i.e. Tris Hcl (pH 8.8) (6 ml), SDS 10% (0.300 ml), ammonium per sulphate 5% (0.150 ml), N, N, N, N – tetra methylene diamide (TEMED) (0.030 ml) and distilled water (13.520 ml) and then it was casted on the gel casting system. The stacking solution was formed by adding 30% acrylamide stock (1.66 ml), stacking gel buffer i.e. Tris Hcl (pH 8.8) (1.38 ml), SDS 10% (100  $\mu\text{l}$ ), ammonium per sulphate 5% (100  $\mu\text{l}$ ), TEMED (10  $\mu\text{l}$ ) and distilled water (6.96 ml). Then the stacking solution was layered over the separating gel after inserting a comb and was allowed to polymerise. Protein samples equivalent to 200  $\mu\text{g}$  each were mixed with equal volume of sample buffer [0.5M Tris Hcl (pH 6.8) 2.5 ml,  $\beta$ -Mercapto ethanol 2.5 ml (25%), glycerol 2.5 ml (25%), Bromophenol blue (1%) 1.25 ml, and SDS 1.25 ml] and heated at 50°C for one minute. After cooling to room temperature, the samples were centrifuged at 1000 rpm for 2 min. The supernatant was loaded on the gel and electrophoresis was carried out at 20° C. The gel was stained for 4 hours with staining solution (0.25g of Coomassie brilliant blue R-250 (Sigma) in 100 ml of 50% methanol containing 7% (V/V) acetic acid.). It was then destained with destaining solution (100 ml methanol, 100 ml glacial acetic acid and volume made upto 1.0 L with distilled water) till the background was colourless and the bands became clearly visible. The destained gel was preserved in 7% acetic acid solution. The gels were placed on Gel Documentation System (Fire Reader-Uvtec, Cambridge, UK) for assessment of banding pattern and then photographed. The presence and absence of polypeptide bands were scored as 1 and 0 respectively to determine variation among various zinc treatments. The relative

mobility of each polypeptide band was calculated using software of the Gel doc system. The molecular weights of the dissociated polypeptides were determined by using molecular weight marker of protein standards which consisted seven standard proteins of known molecular weight i.e. lysozyme (14.3 kDa), soybean trypsin inhibitor (20.1 kDa), carbonic anhydrase (29 kDa), ovalbumin (43 kDa), bovine serum albumin (66 kDa) and phosphorylase-b (97.4 kDa).

The treatments were categorized according to polypeptide banding pattern of soluble protein after Zn-treatments. The binary data matrix for presence (1) / absence (0) of polypeptide bands were analysed using NTSYS Software programme (NTSYSpc2.02e) to estimate Jaccard's similarity co-efficient values (Jaccard, 1908) and clustering of treatments (dendrogram) was carried out using Unweighted Paired Group Method with Arithmetic means (UPGMA)-phenograms (Sokal and Michener, 1958) employing Sequential Agglomerative Hierarchic and Non-overlapping clustering (SAHN).

### **3.8 Statistical analysis**

The data collected on WBPH population, plant growth parameters and yield attributing traits from the field experiment conducted over seasons were subjected to square root transformation wherever required and then the data were analysed following the RBD procedure laid out by Gomez and Gomez (1984). The data recorded from various experiments of pot culture trial were subjected to angular transformation wherever needed and after that the data were analysed following the CRBD procedure (Gomez and Gomez, 1984). All the parameters relating to biochemical composition and nutrient content of rice plants were also analysed through CRBD procedures. The treatment variations were tested for significance by 'F' test. The standard error of means ( $SE_{(m)\pm}$ ) and critical difference (CD) at 5% level of significance were calculated following the standard procedure and treatment means were compared using CDs. Simple correlation coefficient (r values) were determined between zinc content of the plant samples with plant growth parameters, WBPH population and biochemical parameters as well as population build-up of WBPH with various plant biochemical parameters (Panse and Sukhatme, 1967).

# RESULTS

The field reaction of various zinc supplements on the incidence of white backed plant hopper (WBPH) on rice was studied during three seasons *viz.*, *kharif*, 2016, summer, 2016-17 and *kharif*, 2017. Various pot culture and laboratory analysis works were undertaken during *kharif*, 2017. The data generated from the field study as well as pot culture and laboratory studies are presented hereunder under different heads and sub-heads.

## **4.1 Effect of zinc fertilizer on WBPH incidence in rice (field experiment)**

### **4.1.1 Effect of zinc on the incidence of WBPH during *kharif*, 2016**

The data presented in Table 4 revealed that at 40 DAT, the control treatment supported only 5.96 insects/hill which was significantly different from rest other treatments that did not vary among themselves. At 50 DAT, there was marked increase in insect number in all the treatment. Least population of WBPH was evident in T<sub>6</sub> (7.12 insects/hill) which was at par with T<sub>7</sub> (8.70 insects/hill) and T<sub>8</sub> (8.90 insects/hill). In rest of the treatments, the WBPH number varied from 11.46 in T<sub>5</sub> to 14.57 in T<sub>2</sub> having no significant difference between themselves. However, the control treatment harboured 26.13 nymphs/hill which was significantly different from rest of the treatments. At 60 DAT, maximum incidence of WBPH was visualized (T<sub>9</sub> with 44.66 insects/hill). The treatment T<sub>6</sub> encountered 19.67 insects/hill which was at par with T<sub>7</sub> (23.43 insects/hill). The next better treatment was T<sub>8</sub> (29.86 insects/hill) which remained at par with rest of the treatments. But the control treatment that retained 44.66 insects/hill was completely different from all other test treatments. At 70 DAT, there was a decline in WBPH population in all the treatments including control irrespective of the dose and kind of zinc supplementation. Least number of WBPH was witnessed in T<sub>6</sub> (12.53 insects/hill) which was at par with most of the treatments except for T<sub>2</sub> (20.26 insects/hill). The population of WBPH further decreased at 80 DAT. The treatment T<sub>6</sub> (3.53 insects/hill) was the best treatment for recording least population and remained at par with T<sub>7</sub>, T<sub>8</sub> and T<sub>5</sub> treatments, whereas, T<sub>1</sub> and T<sub>2</sub> treatments supported 8.90 and 9.26 insects/hill at this stage. The control treatment supported 14.06 insects/hill which was statistically different from all other treatments.

**Table 4. Effect of zinc on the incidence of WBPH in rice during *kharif*, 2016**

Treatment	Mean WBPH population (numbers/hill)				
	40 DAT	50 DAT	60 DAT	70 DAT	80 DAT
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	3.20 (1.91) <sup>b</sup>	13.93 (3.79) <sup>b</sup>	28.24 (5.36) <sup>bc</sup>	17.90 (4.28) <sup>bc</sup>	8.90 (3.06) <sup>b</sup>
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	3.43 (1.98) <sup>b</sup>	14.57 (3.88) <sup>b</sup>	27.72 (5.30) <sup>bc</sup>	20.26 (4.50) <sup>ab</sup>	9.26 (3.10) <sup>b</sup>
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	3.13 (1.90) <sup>b</sup>	13.26 (3.71) <sup>b</sup>	33.93 (5.84) <sup>b</sup>	17.06 (4.16) <sup>bc</sup>	8.06 (2.88) <sup>bc</sup>
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	2.93 (1.81) <sup>b</sup>	13.20 (3.69) <sup>b</sup>	33.00 (5.72) <sup>b</sup>	15.56 (4.00) <sup>bc</sup>	6.56 (2.66) <sup>bcd</sup>
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	2.76 (1.79) <sup>b</sup>	11.46 (3.46) <sup>bc</sup>	31.60 (5.64) <sup>bc</sup>	15.23 (3.93) <sup>bc</sup>	5.93 (2.52) <sup>bcd</sup>
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	2.26 (1.66) <sup>b</sup>	7.12 (2.76) <sup>d</sup>	19.67 (4.48) <sup>d</sup>	12.53 (3.61) <sup>c</sup>	3.53 (2.00) <sup>d</sup>
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	2.40 (1.67) <sup>b</sup>	8.70 (2.99) <sup>cd</sup>	23.43 (4.88) <sup>cd</sup>	13.60 (3.74) <sup>bc</sup>	4.60 (2.25) <sup>cd</sup>
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	2.41 (1.70) <sup>b</sup>	8.90 (3.05) <sup>cd</sup>	29.86 (5.48) <sup>bc</sup>	14.13 (3.81) <sup>bc</sup>	5.13 (2.35) <sup>cd</sup>
T <sub>9</sub> : Control	5.96 (2.54) <sup>a</sup>	26.13 (5.16) <sup>a</sup>	44.66 (6.72) <sup>a</sup>	32.06 (5.68) <sup>a</sup>	14.06 (3.80) <sup>a</sup>
SE <sub>(m)</sub> ±	0.128	0.178	0.277	0.256	0.220
C.D.(0.05)	0.38	0.53	0.83	0.77	0.66

Figures in parentheses are  $\sqrt{x+0.5}$  transformed values

Means followed by the same letter are not significantly different from each other

#### **4.1.2 Effect of zinc on the incidence of WBPH during summer, 2016-17**

It was evident from Table 5 that at 40 DAT, the WBPH population was very low; T<sub>9</sub> being highest with a mean population of 2.76 nymphs/hill, whereas, in rest of the treatments, the population recorded was less than one. However, at 50 DAT, the control treatment inhabited 9.20 insects/hill which was significantly highest and different from rest of the treatments. At this stage, the treatment T<sub>6</sub> recorded a mean number of 3.38 insects/hill which was almost homogenous with all other test treatments. Variation in WBPH population was marked at 60 DAT where T<sub>6</sub> harboured a mean of 5.24 insects/hill which remained at par with T<sub>7</sub>, T<sub>8</sub>, T<sub>5</sub>, T<sub>4</sub> and T<sub>3</sub> treatments. The treatments T<sub>1</sub> and T<sub>2</sub> recorded 8.19 and 8.37 insects/hill, respectively indicating their relatively less efficacy. At 70 DAT, the trend as witnessed at 60 DAT was almost similar. The treatment T<sub>6</sub> retained only 2.97 insects/hill which was at par with all other treatments except for T<sub>1</sub> (5.94 insects/hill) and T<sub>2</sub> (5.97 insects/hill). At this stage the control treatment (T<sub>9</sub>) registered the highest population (9.71/hill) which was significantly different from rest of the treatments. At 80 DAT, the treatment T<sub>7</sub> (0.17 insects/hill) was the best treatment which did not vary significantly from T<sub>4</sub> (0.45/hill). The next better treatment was T<sub>1</sub> (0.89/hill) which was statistically at par with T<sub>8</sub> (1.01 insects/hill), T<sub>6</sub> (1.02 insects/hill), T<sub>5</sub> (1.11 insects/hill) and T<sub>2</sub> (1.14 insects/hill). However, all the treatments tested were significantly superior to control treatment (5.47 insects/hill).

#### **4.1.3 Effect of zinc on the incidence of WBPH during *kharif*, 2017**

The data depicted in Table 6 envisaged that the WBPH population varied significantly between the treatments at 40 DAT. Lowest WBPH number (2.23/hill) was observed in T<sub>6</sub> which was at par with T<sub>7</sub> (2.98 insects/hill), T<sub>8</sub> (3.46 insects/hill) and T<sub>5</sub> (4.01 insects/hill), respectively. The other treatments contained more insects comparatively, whereas, the control treatment had a mean of 9.41 insects/hill which was significantly quite different from all other treatments. At 50 DAT, it can be seen that the treatment T<sub>6</sub> supported 5.68 insects/hill which was at par with T<sub>7</sub> and T<sub>8</sub> treatments in which the WBPH population ranged from 6.09 to 8.53 per hill. Other treatments *viz.*, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> harboured 10.15 to 13.80 insects/hill without any significant difference between themselves. But the control treatment that registered 19.73 insects/hill was completely different from rest of the treatments.

**Table 5. Effect of zinc on the incidence of WBPH in rice during summer, 2016-17**

Treatment	Mean WBPH population (numbers/hill)				
	40 DAT	50 DAT	60 DAT	70 DAT	80 DAT
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	0.90 (1.18) <sup>b</sup>	4.70 (2.28) <sup>b</sup>	8.19 (2.95) <sup>b</sup>	5.94 (2.53) <sup>b</sup>	0.89 (1.17) <sup>cd</sup>
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	0.95 (1.20) <sup>b</sup>	4.77 (2.28) <sup>b</sup>	8.37 (2.97) <sup>b</sup>	5.97 (2.53) <sup>b</sup>	1.14 (1.27) <sup>bc</sup>
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	0.86 (1.17) <sup>b</sup>	4.56 (2.25) <sup>b</sup>	7.46 (2.82) <sup>bc</sup>	4.85 (2.30) <sup>bc</sup>	1.57 (1.43) <sup>b</sup>
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	0.80 (1.14) <sup>b</sup>	4.52 (2.23) <sup>b</sup>	7.19 (2.76) <sup>bc</sup>	4.27 (2.18) <sup>bc</sup>	0.45 (0.97) <sup>de</sup>
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	0.78 (1.13) <sup>b</sup>	4.21 (2.16) <sup>b</sup>	6.84 (2.70) <sup>bc</sup>	4.10 (2.14) <sup>bc</sup>	1.11 (1.27) <sup>bc</sup>
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water) twice at 30 and 45 DAT	0.74 (1.11) <sup>b</sup>	3.38 (1.97) <sup>b</sup>	5.24 (2.34) <sup>c</sup>	2.97 (1.86) <sup>c</sup>	1.02 (1.23) <sup>bc</sup>
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	0.76 (1.12) <sup>b</sup>	3.75 (2.06) <sup>b</sup>	5.73 (2.49) <sup>bc</sup>	3.81 (2.07) <sup>bc</sup>	0.17 (0.82) <sup>e</sup>
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	0.76 (1.12) <sup>b</sup>	3.94 (2.10) <sup>b</sup>	6.40 (2.61) <sup>bc</sup>	3.99 (2.09) <sup>bc</sup>	1.01 (1.23) <sup>bc</sup>
T <sub>9</sub> : Control	2.76 (1.80) <sup>a</sup>	9.20 (3.08) <sup>a</sup>	14.13 (3.81) <sup>a</sup>	9.71 (3.19) <sup>a</sup>	5.47 (2.44) <sup>a</sup>
SE <sub>(m)</sub> ±	0.057	0.135	0.187	0.161	0.066
C.D.(0.05)	0.17	0.40	0.56	0.48	0.20

Figures in parentheses are  $\sqrt{x+0.5}$  transformed values

Means followed by the same letter are not significantly different from each other

**Table 6. Effect of zinc on the incidence of WBPH population in rice during *kharif*, 2017**

Treatment	Mean WBPH population (numbers/hill)				
	40 DAT	50 DAT	60 DAT	70 DAT	80 DAT
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	6.08 (2.56) <sup>ab</sup>	13.80 (3.77) <sup>b</sup>	14.21 (3.82) <sup>bc</sup>	6.02 (2.54) <sup>b</sup>	2.05 (1.59) <sup>bc</sup>
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	6.21 (2.58) <sup>ab</sup>	12.99 (3.66) <sup>b</sup>	16.44 (4.11) <sup>b</sup>	5.13 (2.37) <sup>bc</sup>	2.11 (1.59) <sup>bc</sup>
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	5.36 (2.38) <sup>bc</sup>	10.76 (3.35) <sup>bc</sup>	11.92 (3.52) <sup>bcd</sup>	4.95 (2.33) <sup>bc</sup>	2.20 (1.63) <sup>b</sup>
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	5.11 (2.33) <sup>bc</sup>	10.15 (3.26) <sup>bcd</sup>	12.85 (3.65) <sup>bcd</sup>	4.63 (2.25) <sup>bcd</sup>	1.13 (1.28) <sup>cd</sup>
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	4.01 (2.10) <sup>bcd</sup>	8.53 (3.00) <sup>cd</sup>	11.22 (3.41) <sup>bcd</sup>	3.61 (2.02) <sup>cde</sup>	1.21 (1.30) <sup>cd</sup>
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	2.23 (1.63) <sup>d</sup>	5.68 (2.47) <sup>e</sup>	9.21 (3.09) <sup>d</sup>	2.15 (1.61) <sup>e</sup>	0.70 (1.09) <sup>d</sup>
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	2.98 (1.80) <sup>cd</sup>	6.09 (2.55) <sup>e</sup>	10.53 (3.31) <sup>cd</sup>	2.93 (1.84) <sup>de</sup>	1.32 (1.35) <sup>bcd</sup>
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	3.46 (1.98) <sup>bcd</sup>	6.89 (2.72) <sup>de</sup>	10.98 (3.38) <sup>cd</sup>	4.14 (2.15) <sup>bcd</sup>	1.55 (1.42) <sup>bc</sup>
T <sub>9</sub> : Control	9.41 (3.13) <sup>a</sup>	19.73 (4.48) <sup>a</sup>	24.18 (4.93) <sup>a</sup>	9.52 (3.16) <sup>a</sup>	4.73 (2.28) <sup>a</sup>
SE <sub>(m)</sub> ±	0.203	0.195	0.241	0.143	0.104
C.D.(0.05)	0.61	0.58	0.72	0.43	0.31

Figures in parentheses are  $\sqrt{x+0.5}$  transformed values

Means followed by the same letter are not significantly different from each other

At 60 DAT, it was observed that T<sub>6</sub> had the lowest WBPH population (9.21 insects/hill) which remained at par with T<sub>7</sub>, T<sub>8</sub>, T<sub>5</sub>, T<sub>3</sub> and T<sub>4</sub> treatments which retained the WBPH between 10.53 to 12.85 insects/hill. The treatments T<sub>1</sub> and T<sub>2</sub> housed comparatively more population (14.21 to 16.44 insects/hill). At this time, the control treatment possessed 24.18 insects/hill which was significantly different from the rest of the treatments. At 70 DAT, the WBPH population declined abruptly in all the treatments including control. The control treatments that supported 9.52 insects/hill was significantly different from rest of the treatments. At 80 DAT, the control treatment supported only a mean of 4.73 insects/hill which was also completely different from rest of the treatments. At this period, T<sub>6</sub> was the best treatment which supported a mean of 0.70 insects/hill and was at par with T<sub>4</sub> (1.13/hill), T<sub>5</sub> (1.21/hill) and T<sub>7</sub> (1.32 insects/hill), respectively.

#### **4.1.4 Effect of zinc on the incidence of WBPH during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (pooled)**

The pooled data for the above three test seasons has been depicted in Table 7. It can be perused from the Table 7 that at 60 DAT, maximum WBPH activity was witnessed (27.66 insects/hill) in T<sub>9</sub> which was statistically different from all other treatments imposed. At this period, T<sub>6</sub> performed best having the least WBPH population (11.37 insects/hill) which was at par with T<sub>7</sub> (13.23 insects/hill). Rest of the treatments supported 15.75 insects/hill in T<sub>8</sub> to as high as 17.77 insects/hill in T<sub>3</sub>. The overall trend of WBPH activity at 70 DAT was almost the same as happened at 60 DAT but at this stage, there was reduction in pest activity. Towards 80 DAT, the pest activity was highly diminished in all the zinc supplemented treatments (1.75 in T<sub>6</sub> to 4.17 in T<sub>2</sub>) but the average population in control was 8.09 insects/hill which was a little lower than ETL.

As regards to the mean performance, it was evident that the treatment T<sub>6</sub> was the best treatment which only harboured a mean of 5.23 insects/hill, closely followed by T<sub>7</sub> (6.05/hill) and T<sub>8</sub> (6.90 /hill). These three treatments caused 66.15, 60.84 and 55.34 per cent reduction in WBPH population, respectively when compared with the control. While the treatment T<sub>5</sub> caused 51.39 per cent reduction in WBPH population, at the same time other treatments (T<sub>1</sub> to T<sub>4</sub>) caused 39.87 to 47.18 per cent reduction in WBPH population.

**Table 7. Effect of zinc on the incidence of WBPH in rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (pooled)**

Treatment	WBPH population (numbers/hill)					Overall mean	% reduction over control
	40 DAT	50 DAT	60 DAT	70 DAT	80 DAT		
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	3.39 (1.88) <sup>bc</sup>	10.81 (3.28) <sup>b</sup>	16.88 (4.04) <sup>b</sup>	9.95 (3.12) <sup>b</sup>	3.95 (1.94) <sup>bc</sup>	9.00	41.75
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	3.53 (1.92) <sup>b</sup>	10.78 (3.28) <sup>b</sup>	17.51 (4.13) <sup>b</sup>	10.45 (3.13) <sup>b</sup>	4.17 (1.99) <sup>b</sup>	9.29	39.87
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	3.12 (1.81) <sup>bcd</sup>	9.53 (3.10) <sup>bc</sup>	17.77 (4.06) <sup>b</sup>	8.95 (2.93) <sup>bc</sup>	3.94 (1.98) <sup>b</sup>	8.66	43.95
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	2.95 (1.76) <sup>bcd</sup>	9.29 (3.06) <sup>bc</sup>	17.68 (4.04) <sup>b</sup>	8.15 (2.81) <sup>cd</sup>	2.71 (1.63) <sup>de</sup>	8.16	47.18
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	2.52 (1.67) <sup>cdef</sup>	8.07 (2.87) <sup>cd</sup>	16.55 (3.92) <sup>bc</sup>	7.65 (2.70) <sup>cd</sup>	2.75 (1.70) <sup>cd</sup>	7.51	51.39
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	1.74 (1.47) <sup>f</sup>	5.39 (2.40) <sup>e</sup>	11.37 (3.30) <sup>d</sup>	5.88 (2.36) <sup>f</sup>	1.75 (1.44) <sup>e</sup>	5.23	66.15
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	2.05 (1.53) <sup>ef</sup>	6.18 (2.53) <sup>e</sup>	13.23 (3.56) <sup>cd</sup>	6.78 (2.55) <sup>def</sup>	2.03 (1.47) <sup>de</sup>	6.05	60.84
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	2.21 (1.60) <sup>def</sup>	6.58 (2.62) <sup>de</sup>	15.75 (3.83) <sup>bc</sup>	7.42 (2.68) <sup>cde</sup>	2.56 (1.67) <sup>de</sup>	6.90	55.34
T <sub>9</sub> : Control	6.04 (2.49) <sup>a</sup>	18.35 (4.24) <sup>a</sup>	27.66 (5.15) <sup>a</sup>	17.09 (3.73) <sup>a</sup>	8.09 (2.84) <sup>a</sup>	15.45	-
SE <sub>(m)</sub> ±	0.086	0.099	0.135	0.106	0.084	-	-
C.D. (0.05)	0.24	0.30	0.38	0.30	0.25	-	-

Figures in parentheses are transformed values

Means followed by the same letter are not significantly different from each other

Hence, from the three seasons data and pooled data (Table 4 to 7), it was inferred that application of zinc in rice regardless of its form and method of application was effective in reducing WBPH population substantially.

## **4.2 Effect of zinc on growth attributes of rice plant**

### **4.2.1 Effect of zinc supplementation on tiller number in rice during *kharif*, 2016**

A perusal of data presented in Table 8 indicated that the tiller number varied significantly between the treatments at 30 DAT, whereas, in all the treatments, the tiller number per hill was less than 10, but at 60 DAT, variation between the treatments was more pronounced. At this stage, maximum tiller (16.4/hill) was witnessed in T<sub>5</sub> and T<sub>2</sub> which was at par with T<sub>7</sub> (16.20/hill), T<sub>8</sub> and T<sub>6</sub> (15.50/hill, each). The control treatment had only 9.20 tillers/hill which was significantly the lowest. At 90 DAT, highest number of tillers was observed in T<sub>5</sub> (15.90/hill) which remained distinctly different from the rest. The next better treatment was T<sub>6</sub> (14.40/hill) which was at par with T<sub>7</sub>, T<sub>2</sub> and T<sub>8</sub>, respectively.

### **4.2.2 Effect of zinc supplementation on tiller number in rice during summer, 2016-17**

The data on tiller number on 30 DAT during summer, 2016-17 presented in Table 9 that the tiller number per hill ranged from as low as 4.40/hill to 6.60/hill within the treatments having significant difference between themselves. At 60 DAT, there was a marked boosting in tiller number. The treatment T<sub>6</sub> had a mean of 14.80 tillers/hill and T<sub>8</sub> also had a similar number of tiller/hill. Both the treatments were at par with T<sub>7</sub>, T<sub>5</sub> and T<sub>2</sub>, whereas, in other treatments the tiller numbers/hill ranged from 10.10 to 12.00 (T<sub>1</sub>). At this stage, the control treatment retained only 8.30 tillers/hill which was significantly lowest among the treatments tested.

### **4.2.3 Effect of zinc supplementation on tiller number in rice during *kharif*, 2017**

Table 10 denotes the data on tiller number during *kharif*, 2017. At 30 DAT, the tiller number varied from as low as 4.80/hill in T<sub>9</sub> to as high as 6.40/hill in T<sub>6</sub> having distinct difference among the treatments. At 60 DAT, there were 16.20 tillers/hill in T<sub>6</sub> which was nearly identical to T<sub>7</sub>, T<sub>5</sub> and T<sub>8</sub> treatments. Other treatments *viz.*, T<sub>1</sub> to T<sub>4</sub> supported 11.80 to 14.00 tillers/hill having difference within themselves, whereas, the control treatment had significantly lowest number of tillers (9.00/hill).

**Table 8. Effect of zinc on number of tillers/hill in rice during *kharif*, 2016**

Treatment	Tillers/hill		
	30 DAT	60 DAT	90 DAT
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	7.50 <sup>ab</sup>	14.60 <sup>b</sup>	11.20 <sup>c</sup>
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	6.90 <sup>c</sup>	16.40 <sup>a</sup>	13.60 <sup>b</sup>
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	5.20 <sup>d</sup>	11.30 <sup>c</sup>	8.60 <sup>d</sup>
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	5.00 <sup>d</sup>	11.60 <sup>c</sup>	11.00 <sup>c</sup>
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	7.80 <sup>a</sup>	16.40 <sup>a</sup>	15.90 <sup>a</sup>
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	7.20 <sup>bc</sup>	15.50 <sup>ab</sup>	14.40 <sup>b</sup>
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	7.70 <sup>ab</sup>	16.20 <sup>a</sup>	14.00 <sup>b</sup>
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	7.60 <sup>ab</sup>	15.50 <sup>ab</sup>	13.40 <sup>b</sup>
T <sub>9</sub> : Control	5.00 <sup>d</sup>	9.20 <sup>d</sup>	7.50 <sup>d</sup>
SE <sub>(m)</sub> ±	0.183	0.409	0.434
C.D.(0.05)	0.55	1.22	1.30

Means followed by the same letter are not significantly different from each other

**Table 9. Effect of zinc on number of tillers/hill in rice during summer, 2016-17**

Treatment	Tillers/hill		
	30 DAT	60 DAT	90 DAT
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	6.10 <sup>a</sup>	12.00 <sup>b</sup>	10.60 <sup>c</sup>
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	5.90 <sup>a</sup>	13.80 <sup>a</sup>	13.70 <sup>ab</sup>
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	4.50 <sup>b</sup>	10.10 <sup>c</sup>	8.80 <sup>de</sup>
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	4.40 <sup>b</sup>	11.30 <sup>b</sup>	10.00 <sup>cd</sup>
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	6.60 <sup>a</sup>	13.90 <sup>a</sup>	12.70 <sup>b</sup>
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water)twice at 30 and 45 DAT	6.10 <sup>a</sup>	14.80 <sup>a</sup>	13.60 <sup>ab</sup>
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha) + Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	6.40 <sup>a</sup>	14.60 <sup>a</sup>	14.50 <sup>a</sup>
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	6.00 <sup>a</sup>	14.80 <sup>a</sup>	13.50 <sup>ab</sup>
T <sub>9</sub> : Control	4.50 <sup>b</sup>	8.30 <sup>d</sup>	7.50 <sup>c</sup>
SE <sub>(m)</sub> ±	0.240	0.343	0.436
C.D.(0.05)	0.72	1.03	1.31

Means followed by the same letter are not significantly different from each other

**Table 10. Effect of zinc on number of tillers/hill in rice during *kharif*, 2017**

Treatment	Tillers/hill		
	30 DAT	60 DAT	90 DAT
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	5.80 <sup>b</sup>	12.50 <sup>c</sup>	10.80 <sup>de</sup>
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	6.00 <sup>ab</sup>	14.00 <sup>b</sup>	13.30 <sup>c</sup>
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	4.70 <sup>c</sup>	11.80 <sup>c</sup>	10.00 <sup>e</sup>
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	4.90 <sup>c</sup>	12.30 <sup>ac</sup>	11.80 <sup>d</sup>
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	5.80 <sup>b</sup>	15.70 <sup>a</sup>	14.20 <sup>abc</sup>
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	6.40 <sup>a</sup>	16.20 <sup>a</sup>	15.20 <sup>a</sup>
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	6.10 <sup>ab</sup>	15.80 <sup>a</sup>	14.90 <sup>ab</sup>
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	6.50 <sup>a</sup>	15.30 <sup>a</sup>	14.10 <sup>bc</sup>
T <sub>9</sub> : Control	4.80 <sup>c</sup>	9.00 <sup>d</sup>	8.70 <sup>f</sup>
SE <sub>(m)</sub> ±	0.189	0.356	0.345
C.D.(0.05)	0.57	1.07	1.03

Means followed by the same letter are not significantly different from each other

#### **4.2.4 Effect of zinc supplementation on tiller number in rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (pooled)**

A perusal of pooled data for three seasons depicted in Table 11 revealed that at 60 DAT (coinciding with maximum WBPH incidence) T<sub>7</sub> had the highest number of tillers (15.53/hill) which was at par with T<sub>6</sub>, T<sub>5</sub> and T<sub>8</sub>. A similar trend was witnessed at 90 DAT. With reference to overall mean performance, it could be visualized that T<sub>7</sub> supported highest number of tillers/hill (12.24) closely followed by T<sub>6</sub> (12.16) and T<sub>5</sub> (12.11). These three treatments witnessed 70.71, 69.59 and 68.90 per cent increase in tiller number as compared to control.

#### **4.2.5 Effect of zinc application on plant height in rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 and pooled over season**

The data on plant height due to influence of zinc fertilizer application during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 and pooled mean has been denoted in Table 12. It was revealed from Table 12 that during *kharif*, 2016, plant height was maximum i.e. 84.00 cm in both T<sub>6</sub> and T<sub>7</sub> which was on a par with all the treatments except control. The control treatment had lowest plant height (74.80 cm). During summer, 2016-17, T<sub>2</sub> produced a plant height of 80.80 cm which was significantly at par with all the treatments (except control). The control treatment witnessed a plant height of 70.20 cm. A perusal of data of *kharif*, 2017, indicated that the treatment T<sub>5</sub> accounted for a plant height of 82.20 cm which was nearly identical to T<sub>6</sub> (82.00 cm), T<sub>7</sub> (82.00 cm) and T<sub>8</sub> (81.20 cm), whereas, in other treatments (T<sub>1</sub> to T<sub>4</sub>) plant height ranged from 77.20 to 80.10 cm having difference within themselves. The control treatment had significantly lowest plant height (72.30 cm). With reference to the pooled mean performance it could be visualized that T<sub>6</sub> produced maximum plant height (81.33 cm) closely followed by T<sub>8</sub> (81.27 cm) and T<sub>7</sub> (81.17 cm). These three treatments witnessed 12.29, 12.20 and 12.07 per cent increase in plant height over control, respectively.

### **4.3 Influence of zinc on yield and yield attributing traits in rice**

#### **4.3.1 Effect of zinc on number of panicle/ m<sup>2</sup> in rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 and pooled over season**

The data on number of panicles per square meter under the influence of zinc fertilization on rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 and pooled performance has been depicted in Table 13. It can be denoted from Table 13 that during

**Table 11. Effect of zinc on number of tillers/hill in rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (pooled)**

Treatment	Tillers/hill			Overall mean	Increase over control (%)
	30 DAT	60 DAT	90 DAT		
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	6.47 <sup>ab</sup>	13.03 <sup>c</sup>	10.87 <sup>d</sup>	10.12	41.14
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	6.27 <sup>b</sup>	14.73 <sup>b</sup>	13.53 <sup>c</sup>	11.51	60.53
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	4.80 <sup>c</sup>	11.07 <sup>e</sup>	9.13 <sup>e</sup>	8.33	16.19
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	4.77 <sup>c</sup>	11.73 <sup>d</sup>	10.93 <sup>d</sup>	9.14	27.47
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	6.73 <sup>a</sup>	15.33 <sup>ab</sup>	14.27 <sup>ab</sup>	12.11	68.90
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	6.57 <sup>ab</sup>	15.50 <sup>a</sup>	14.40 <sup>a</sup>	12.16	69.59
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha) + Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	6.73 <sup>a</sup>	15.53 <sup>a</sup>	14.47 <sup>a</sup>	12.24	70.71
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	6.70 <sup>a</sup>	15.20 <sup>ab</sup>	13.67 <sup>bc</sup>	11.86	65.41
T <sub>9</sub> : Control	4.77 <sup>c</sup>	8.83 <sup>f</sup>	7.90 <sup>f</sup>	7.17	-
SE <sub>(m)</sub> ±	0.119	0.214	0.235	-	-
C.D.(0.05)	0.36	0.64	0.70	-	-

Means followed by the same letter are not significantly different from each other

**Table 12. Effect of zinc on plant height in rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (pooled)**

Treatment	Plant height (cm)			Pooled	Increase over control (%)
	<i>kharif</i> , 2016	summer, 2016-17	<i>kharif</i> , 2017		
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	83.20 <sup>a</sup>	78.80 <sup>a</sup>	80.10 <sup>a</sup>	80.70 <sup>a</sup>	11.42
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	82.70 <sup>a</sup>	80.80 <sup>a</sup>	78.40 <sup>a</sup>	80.63 <sup>a</sup>	11.32
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	80.00 <sup>ab</sup>	75.80 <sup>a</sup>	77.20 <sup>ab</sup>	77.67 <sup>b</sup>	7.23
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	81.10 <sup>a</sup>	77.00 <sup>a</sup>	79.00 <sup>a</sup>	79.03 <sup>ab</sup>	9.11
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	81.30 <sup>a</sup>	79.30 <sup>a</sup>	82.20 <sup>a</sup>	80.93 <sup>a</sup>	11.74
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water) twice at 30 and 45 DAT	84.00 <sup>a</sup>	78.00 <sup>a</sup>	82.00 <sup>a</sup>	81.33 <sup>a</sup>	12.29
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	84.00 <sup>a</sup>	77.50 <sup>a</sup>	82.00 <sup>a</sup>	81.17 <sup>a</sup>	12.07
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	82.50 <sup>a</sup>	80.10 <sup>a</sup>	81.20 <sup>a</sup>	81.27 <sup>a</sup>	12.20
T <sub>9</sub> : Control	74.80 <sup>b</sup>	70.20 <sup>b</sup>	72.30 <sup>b</sup>	72.43 <sup>c</sup>	-
SE <sub>(m)</sub> ±	1.758	1.813	1.942	0.983	-
C.D.(0.05)	5.27	5.44	5.82	2.78	-

Means followed by the same letter are not significantly different from each other

**Table 13. Effect of zinc on number of panicle/ m<sup>2</sup> in rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (pooled)**

Treatment	Panicle/ m <sup>2</sup>			Pooled	Increase over control (%)
	<i>kharif</i> , 2016	summer, 2016-17	<i>kharif</i> , 2017		
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	281.00 <sup>a</sup>	249.00 <sup>c</sup>	267.00 <sup>b</sup>	265.67 <sup>c</sup>	55.66
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	289.00 <sup>a</sup>	265.00 <sup>bc</sup>	280.00 <sup>ab</sup>	278.00 <sup>bc</sup>	62.89
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	199.00 <sup>b</sup>	198.00 <sup>d</sup>	203.00 <sup>d</sup>	200.00 <sup>e</sup>	17.19
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	203.00 <sup>b</sup>	218.00 <sup>d</sup>	230.00 <sup>c</sup>	217.00 <sup>d</sup>	27.15
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	286.00 <sup>a</sup>	291.00 <sup>a</sup>	290.00 <sup>ab</sup>	289.00 <sup>ab</sup>	69.33
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	297.00 <sup>a</sup>	289.00 <sup>ab</sup>	289.00 <sup>ab</sup>	291.67 <sup>ab</sup>	70.90
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha) + Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	285.00 <sup>a</sup>	295.00 <sup>a</sup>	270.00 <sup>b</sup>	283.33 <sup>ab</sup>	66.01
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	302.00 <sup>a</sup>	288.00 <sup>ab</sup>	294.00 <sup>a</sup>	294.67 <sup>a</sup>	72.65
T <sub>9</sub> : Control	176.00 <sup>b</sup>	170.00 <sup>e</sup>	166.00 <sup>e</sup>	170.67 <sup>f</sup>	-
SE <sub>(m)</sub> ±	9.289	8.253	7.752	5.090	-
C.D.(0.05)	27.84	24.74	23.24	14.38	-

Means followed by the same letter are not significantly different from each other

*kharif*, 2016, number of panicles/ m<sup>2</sup> was highest in T<sub>8</sub> (302) which was at par with many treatments *viz.*, T<sub>2</sub> (289), T<sub>6</sub> (297), T<sub>5</sub> (286), T<sub>7</sub> (285) and T<sub>1</sub> (281). The control treatment witnessed 176 panicles/ m<sup>2</sup> which was significantly lowest among the treatments. During summer, 2016-17, T<sub>7</sub> had the highest number of panicles (295) which was at par with T<sub>5</sub> (291), T<sub>6</sub> (289) and T<sub>8</sub> (288). The control treatment had the lowest number of panicles/m<sup>2</sup> (170) which was distinctly different from all other treatments except T<sub>3</sub> (198). A perusal of data for *kharif*, 2017 revealed that the treatments T<sub>8</sub> accounted for 294 panicles/m<sup>2</sup> which remained on a par with T<sub>5</sub> (290), T<sub>6</sub> (289) and T<sub>2</sub> (280). The performance of the treatments over 3 years was consistent. In all the years, significantly higher number of panicles were observed in zinc treatments than the control treatment. However, within the zinc treatments, the performance of T<sub>3</sub> and T<sub>4</sub> was a little bit poor in all the three years. As regards to the pooled mean, it was also witnessed that T<sub>8</sub> had the highest mean number of panicles (294.67/m<sup>2</sup>) followed by rest of treatments except for T<sub>1</sub>, T<sub>3</sub> and T<sub>4</sub> treatments. It was inferred from the three season data that there was 17.19 to 72.65 per cent enhancement of the panicle number/ m<sup>2</sup> in different zinc treatments as compared to no treatment.

#### **4.3.2 Effect of zinc supplementation on number of grains/ panicle in rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 and pooled over season**

The data on number of grains per panicle due to application of zinc fertilizer during *kharif*, 2016, summer, 2016-17, *kharif*, 2017 and its pooled mean has been presented in Table 14. It can be inferred from Table 14 that during *kharif*, 2016, number of grains per panicle was highest in T<sub>6</sub> (136.70) which was at par with various treatments *viz.*, T<sub>2</sub> (125.70), T<sub>5</sub> (129.80), T<sub>7</sub> (134.80) and T<sub>8</sub> (130.80), whereas, the control treatment had significantly lowest number of grains per panicle (110.20). During summer, 2016-17, T<sub>6</sub> had highest numbers of grains per panicle (143.40) which was at par with T<sub>2</sub> (137.50), T<sub>5</sub> (135.80), T<sub>7</sub> (138.00), T<sub>8</sub> (136.00), respectively. The control treatment produced lowest grains per panicle (114.50). A perusal of data of *kharif*, 2017, revealed that the treatment T<sub>5</sub> accounted for 130.50 grains per panicle which remained at par with T<sub>2</sub> (120.00), T<sub>7</sub> (129.00), T<sub>6</sub> (126.70) and T<sub>8</sub> (127.40). The control treatment witnessed lowest grains per panicle (104.60) which was significantly lowest among the treatments. As regards to pooled mean performance, it was observed that T<sub>6</sub> obtained the highest grains per panicle (135.60) closely followed by T<sub>7</sub> (133.93), T<sub>5</sub> (132.03) and T<sub>8</sub> (131.40). These four treatments caused 23.53, 22.01,

**Table 14. Effect of zinc on number of number of grains/panicle in rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (pooled)**

Treatment	Grains/panicle			Pooled	Increase over control (%)
	<i>kharif</i> , 2016	summer, 2016-17	<i>kharif</i> , 2017		
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	122.50 <sup>bcd</sup>	130.80 <sup>bc</sup>	115.60 <sup>bc</sup>	122.97 <sup>c</sup>	12.03
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	125.70 <sup>abcd</sup>	137.50 <sup>ab</sup>	120.00 <sup>ab</sup>	127.73 <sup>bc</sup>	16.36
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	118.50 <sup>cde</sup>	116.70 <sup>d</sup>	110.30 <sup>cd</sup>	115.17 <sup>d</sup>	4.92
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	115.00 <sup>de</sup>	119.00 <sup>cd</sup>	113.60 <sup>cd</sup>	115.87 <sup>d</sup>	5.56
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	129.80 <sup>abc</sup>	135.80 <sup>ab</sup>	130.50 <sup>a</sup>	132.03 <sup>ab</sup>	20.28
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	136.70 <sup>a</sup>	143.40 <sup>a</sup>	126.70 <sup>ab</sup>	135.60 <sup>a</sup>	23.53
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	134.80 <sup>ab</sup>	138.00 <sup>ab</sup>	129.00 <sup>a</sup>	133.93 <sup>ab</sup>	22.01
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	130.80 <sup>abc</sup>	136.00 <sup>ab</sup>	127.40 <sup>a</sup>	131.40 <sup>ab</sup>	19.70
T <sub>9</sub> : Control	110.20 <sup>e</sup>	114.50 <sup>d</sup>	104.60 <sup>d</sup>	109.77 <sup>d</sup>	-
SE <sub>(m)</sub> ±	4.243	4.158	3.800	2.194	-
C.D.(0.05)	12.72	12.46	11.39	6.20	-

Means followed by the same letter are not significantly different from each other

20.28, 19.70 per cent increase in grain numbers/ panicle when compared with control, respectively. At the same time other treatments (T<sub>1</sub> to T<sub>4</sub>) increased the grains per panicle from 4.92 to 16.36 per cent. Hence, from three seasons' data and pooled mean, it was concluded that zinc application must have exercised some influence in increasing number of grains per panicle substantially.

### **4.3 Effect of zinc application on grain yield in rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 and pooled over season**

The data on grain yield due to application of zinc fertilizer during *kharif*, 2016 summer, 2016-17 and *kharif*, 2017 and pooled mean over the seasons has been depicted in Table 15. It is indicated from the Table 15 that during *kharif*, 2016, the grain yield was highest in T<sub>7</sub> (37.27 q/ha) which was significantly different from all other treatments. At the same time other treatments T<sub>5</sub>, T<sub>6</sub> and T<sub>8</sub> produced a grain yield of 31.55, 33.51 and 28.12 q/ha, respectively, whereas, the control treatment (21.45 q/ha) was distinctly different being lowest from all other treatments. During summer, 2016-17, T<sub>6</sub> had a maximum grain yield of 36.50 q/ha which was on a par with T<sub>5</sub> (33.49 q/ha), T<sub>7</sub> (34.75 q/ha) and T<sub>8</sub> (32.60 q/ha), whereas, the control treatment produced lowest grain yield (19.92 q/ha). A perusal of data of *kharif*, 2017, showed that the treatment T<sub>6</sub> accounted for grain yield of 31.35 q/ha which was significantly different from all other treatments. The treatments T<sub>7</sub> and T<sub>8</sub> produced a grain yield of 27.52 and 25.70 q/ha, respectively. Rest of the treatment (T<sub>1</sub> to T<sub>4</sub>) varied from 17.59 to 22.18 q/ha. The control treatment witnessed lowest grain yield (16.83 q/ha). In all the three seasons, significantly higher grain yield was observed in zinc treated plot than control one. However, with in the zinc treatment T<sub>6</sub> overall produced higher yield. As regard to the pooled mean, it was also observed that T<sub>6</sub> had the highest grain yield (33.79 q/ha) followed by a number of treatments except for T<sub>1</sub>, T<sub>3</sub> and T<sub>4</sub>. It was traced from all the three season data that there was 16.54 to 74.17 per cent increase in grain yield in different zinc treatments as compared to control.

## **4.4 Pot culture experiment**

### **4.4.1 Antixenosis mechanism induced by zinc**

#### **4.4.1.1 Impact of zinc on alightment of nymphs and adults of WBPH in rice**

The data presented in Table 16 on nymphal orientation on caged rice plants exposed to various zinc treatments focused significant effect of the treatments.

**Table 15. Effect of zinc on grain yield of rice during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (pooled)**

Treatment	Grain yield (q/ha)			Pooled	Increase over control (%)
	<i>kharif</i> , 2016	summer, 2016-17	<i>kharif</i> , 2017		
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	24.50 <sup>de</sup>	28.50 <sup>cd</sup>	19.40 <sup>de</sup>	24.13 <sup>d</sup>	24.38
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	27.34 <sup>cd</sup>	29.56 <sup>bcd</sup>	22.18 <sup>cd</sup>	26.36 <sup>c</sup>	35.87
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	22.64 <sup>ef</sup>	27.61 <sup>d</sup>	17.59 <sup>de</sup>	22.61 <sup>d</sup>	16.54
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	23.30 <sup>ef</sup>	27.90 <sup>d</sup>	18.10 <sup>e</sup>	23.10 <sup>d</sup>	19.07
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	31.55 <sup>b</sup>	33.49 <sup>ab</sup>	22.56 <sup>cd</sup>	29.20 <sup>b</sup>	50.51
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	33.51 <sup>b</sup>	36.50 <sup>a</sup>	31.35 <sup>a</sup>	33.79 <sup>a</sup>	74.17
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	37.27 <sup>a</sup>	34.75 <sup>a</sup>	27.52 <sup>b</sup>	33.18 <sup>a</sup>	71.03
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	28.12 <sup>c</sup>	32.60 <sup>abc</sup>	25.70 <sup>bc</sup>	28.81 <sup>b</sup>	48.50
T <sub>9</sub> : Control	21.45 <sup>f</sup>	19.92 <sup>e</sup>	16.83 <sup>e</sup>	19.40 <sup>e</sup>	-
SE <sub>(m)</sub> ±	0.973	1.366	1.228	0.693	-
C.D.(0.05)	2.92	4.10	3.68	2.08	-

Means followed by the same letter are not significantly different from each other

**Table 16. Effect of zinc on alightment of nymphs and adults of WBPH in rice**

Treatment	Nymphal alightment at different hours after release* (%)					Adults alightment at different hours after release* (%)				
	6 hour	24 hour	48 hour	72 hour	Mean	6 hour	24 hour	48 hour	72 hour	Mean
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	7.68 <sup>b</sup>	8.11 <sup>bc</sup>	8.16 <sup>cd</sup>	8.25 <sup>cde</sup>	8.05	9.38 <sup>b</sup>	10.09 <sup>b</sup>	11.15 <sup>b</sup>	11.21 <sup>b</sup>	10.46
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	7.96 <sup>b</sup>	8.23 <sup>b</sup>	9.32 <sup>b</sup>	9.53 <sup>b</sup>	8.76	9.73 <sup>b</sup>	10.53 <sup>b</sup>	11.03 <sup>b</sup>	11.32 <sup>b</sup>	10.65
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	6.33 <sup>c</sup>	7.59 <sup>bcd</sup>	8.21 <sup>c</sup>	8.47 <sup>bcd</sup>	7.65	8.25 <sup>c</sup>	9.27 <sup>c</sup>	9.76 <sup>c</sup>	10.12 <sup>bc</sup>	9.35
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	6.15 <sup>c</sup>	7.36 <sup>cd</sup>	8.67 <sup>bc</sup>	8.72 <sup>bc</sup>	7.73	8.17 <sup>c</sup>	9.02 <sup>c</sup>	9.59 <sup>c</sup>	10.19 <sup>bc</sup>	9.24
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	5.21 <sup>d</sup>	6.91 <sup>d</sup>	7.24 <sup>de</sup>	7.44 <sup>cde</sup>	6.70	7.32 <sup>d</sup>	8.13 <sup>d</sup>	8.38 <sup>d</sup>	8.95 <sup>cd</sup>	8.20
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	4.02 <sup>f</sup>	5.33 <sup>e</sup>	6.02 <sup>f</sup>	6.07 <sup>g</sup>	5.36	5.13 <sup>f</sup>	5.85 <sup>f</sup>	6.12 <sup>f</sup>	7.10 <sup>e</sup>	6.05
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	4.32 <sup>ef</sup>	5.48 <sup>e</sup>	6.46 <sup>ef</sup>	6.62 <sup>fg</sup>	5.72	6.51 <sup>e</sup>	7.04 <sup>e</sup>	7.32 <sup>e</sup>	7.16 <sup>e</sup>	7.01
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	5.14 <sup>de</sup>	6.03 <sup>e</sup>	7.11 <sup>e</sup>	7.32 <sup>ef</sup>	6.40	7.09 <sup>de</sup>	7.88 <sup>d</sup>	8.42 <sup>d</sup>	8.09 <sup>de</sup>	7.87
T <sub>9</sub> : Control	10.32 <sup>a</sup>	13.25 <sup>a</sup>	15.89 <sup>a</sup>	19.75 <sup>a</sup>	14.80	13.06 <sup>a</sup>	14.01 <sup>a</sup>	17.26 <sup>a</sup>	18.23 <sup>a</sup>	15.64
SE <sub>(m)</sub> ±	0.282	0.276	0.311	0.379	-	0.251	0.228	0.247	0.481	-
C.D.(0.05)	0.84	0.83	0.93	1.14	-	0.75	0.68	0.74	1.44	-

\*Mean of three replications

Means followed by the same letter are not significantly different from each other

Though the per cent nymphal orientation at 6 hours after exposure was significant, T<sub>6</sub> and T<sub>7</sub> expressed their superiority over others. The control treatment captured 10.32 per cent nymphs, whereas, in other treatments, nymphal attraction was 30-60 per cent less. After 24 hours, the control invited 13.25 per cent nymphs, whereas, in other treatments, nymphal attraction was 30-60 per cent less.

After 48 hours of release, the plants of treatment T<sub>6</sub> was foraged by 6.02 per cent nymphs which was only at par with T<sub>7</sub> (6.46 %). In rest of the treatments, the nymphal orientation varied from 7.11 per cent in T<sub>8</sub> to 9.32 per cent in T<sub>2</sub>, whereas, the corresponding control treatment attracted 15.89 per cent nymphs which was significantly higher than all the treatments. At 72 hours of release, T<sub>6</sub> invited 6.07 per cent nymphs which was only homogenous to T<sub>7</sub>. The per cent attraction of nymphs was found to be more in control (19.75 %). While perusing the mean orientation data, it was evident that the treatment T<sub>6</sub> had least orientation (5.36 %) closely followed by T<sub>7</sub> (5.72 %) and T<sub>8</sub> (6.40 %). The control treatment attracted only a mean of 14.80 per cent nymphs.

The data presented in Table 16 on adult orientation on caged rice plants revealed that after 6 hours of release, the female adult alightment was 5-13 per cent which was significantly lowest among all the treatments. At 24 hours of release, there was marginal increase in per cent alightment of adults in all the treatments; highest being noticed in control (14.01 %). At 48 hours after release, it was observed that the treatment T<sub>6</sub> could attract 6.12 per cent adults, whereas, in other treatments, the per cent adult attraction varied from 7.32 to 11.15 per cent regardless of the treatments, whereas, the control treatment at this stage attracted 17.26 per cent adults which was significantly higher than others. After 72 hours of exposure, it was evident that T<sub>6</sub> retained 7.10 per cent adults which was statistically at par with T<sub>7</sub> (7.16 %) and T<sub>8</sub> (8.09 %). The mean performance revealed that 6.05 per cent adults were attracted to T<sub>6</sub> while 7.01 per cent adults were attracted to T<sub>7</sub> and 7.87 and 8.20 per cent adults were attracted to T<sub>8</sub> and T<sub>5</sub>, respectively. Other treatments caused 9.24 to 10.46 per cent alightment, whereas, the control could attract an average of 15.64 per cent adults.

#### **4.4.1.2 Impact of zinc on oviposition by WBPH females in rice**

The data on number of eggs laid and corresponding per cent of eggs which did not hatch is presented in Table 17. It could be appraised from the Table 17 that while control treatment received 86.20 eggs from a single female remaining statistically different from other; the treatment T<sub>6</sub> received only 34.40 eggs which was completely different from the rest. There did not exist any significant variation between T<sub>8</sub> (45.40) and T<sub>5</sub> (46.80), whereas, the rest of the treatments varied significantly with each other. As regards to the percentage of eggs which failed to hatch, it was apparent that only 8.80 per cent eggs did not hatch in control treatment, whereas, highest percentage of unhatched eggs was noticed in T<sub>6</sub> (31.68 %). The treatments T<sub>1</sub> and T<sub>2</sub> did not differ from each other while T<sub>3</sub> and T<sub>4</sub> also did not differ from each other.

#### **4.4.2 Antibiosis mechanism induced by zinc**

##### **4.4.2.1 Impact of zinc application on different antibiosis parameters of WBPH in rice**

The data on nymphal survival, nymphal development, sex ratio, growth index, adult longevity, population build up, feeding potential and weight gain of WBPH under the influence of zinc fertilization has been presented in Table 18. A perusal of data revealed that nymphal survival of WBPH was adversely affected as only 44 per cent nymphs survived on T<sub>6</sub> plants which was statistically different from rest other treatments. The next better treatment was T<sub>7</sub> (51 %) which was at par with T<sub>5</sub> (55 %) and T<sub>8</sub> (56 %). The treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> sustained 71, 73, 67 and 69 per cent survival of the nymphs, respectively which did not vary among themselves. But the control treatment registered 92 per cent survival which was significantly highest.

As regards to nymphal developmental period, it was also witnessed that nymphal developmental period was highest (17.30 days) in T<sub>6</sub> which was only at par with T<sub>7</sub> (17.00 days). All the zinc supplemented treatments extended the nymphal developmental period as compared to control (13.90 days) except for T<sub>1</sub> (14.00 days) which was at par with control.

With respect to sex ratio, it was indicated that the F:M ratio was less than 1 in T<sub>6</sub> and T<sub>7</sub> without any difference between them, whereas, in all other zinc treatments, the ratio was though more than one, yet, statistical difference existed between themselves. Only the treatment T<sub>1</sub> was observed to be similar with control statistically in which the F : M ratio was 1.54 and 1.93, respectively.

**Table 17. Effect of zinc on oviposition by WBPH females in rice**

Treatment	Number of eggs laid*	Unhatched eggs* (%)
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	61.20 <sup>b</sup>	22.30 <sup>e</sup>
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	59.60 <sup>c</sup>	22.10 <sup>e</sup>
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	58.80 <sup>c</sup>	23.20 <sup>d</sup>
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% ( 8g/l of water) twice at 30 and 45 DAT	55.60 <sup>d</sup>	23.60 <sup>d</sup>
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	46.80 <sup>e</sup>	25.90 <sup>c</sup>
T <sub>6</sub> : Zn EDTA basal (40 kg/ha) + Zn EDTA Foliar spray@ 0.8% (8 g/l of water) twice at 30 and 45 DAT	34.40 <sup>g</sup>	31.68 <sup>a</sup>
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	38.60 <sup>f</sup>	28.35 <sup>b</sup>
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	45.40 <sup>e</sup>	26.77 <sup>c</sup>
T <sub>9</sub> : Control	86.20 <sup>a</sup>	8.80 <sup>f</sup>
SE <sub>(m)</sub> ±	0.542	0.303
C.D.(0.05)	1.56	0.87

\*Mean of five replications

Means followed by the same letter are not significantly different from each other

**Table 18. Impact of zinc application on various antibiosis parameters of WBPH in rice**

Treatment	Nymphal survival* (%)	Nymphal period* (days)	Sex ratio* (Female: male)	Growth Index*	Mean adult longevity* (days)		Population build up*	Area of honeydew excreted by 5 females in 24 hours (mm <sup>2</sup> )*	Mean weight gain by WBPH 48 hours after feeding (mg) **	
					Male	Female			Female	Male
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	71.00 (57.43) <sup>bc</sup>	14.00 <sup>e</sup>	1.54 <sup>ab</sup>	5.07 <sup>b</sup>	12.40 <sup>b</sup>	16.20 <sup>b</sup>	53.60 <sup>b</sup>	255.80 <sup>c</sup>	0.05 <sup>b</sup>	0.005 <sup>b</sup>
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	73.00 (58.68) <sup>b</sup>	14.30 <sup>e</sup>	1.62 <sup>ab</sup>	5.10 <sup>b</sup>	11.80 <sup>bc</sup>	15.60 <sup>bc</sup>	51.20 <sup>bc</sup>	262.20 <sup>b</sup>	0.05 <sup>b</sup>	0.006 <sup>b</sup>
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	67.00 (54.95) <sup>c</sup>	15.60 <sup>cd</sup>	1.23 <sup>bc</sup>	4.29 <sup>c</sup>	11.00 <sup>d</sup>	14.80 <sup>cd</sup>	49.80 <sup>bc</sup>	256.60 <sup>c</sup>	0.05 <sup>b</sup>	0.004 <sup>b</sup>
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	69.00 (56.29) <sup>bc</sup>	15.20 <sup>d</sup>	1.19 <sup>bc</sup>	4.54 <sup>bc</sup>	11.40 <sup>cd</sup>	15.20 <sup>c</sup>	49.00 <sup>cd</sup>	254.00 <sup>d</sup>	0.03 <sup>cd</sup>	0.003 <sup>b</sup>
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	55.00 (47.86) <sup>d</sup>	16.50 <sup>b</sup>	1.06 <sup>c</sup>	3.33 <sup>de</sup>	10.20 <sup>e</sup>	14.00 <sup>d</sup>	45.20 <sup>de</sup>	226.60 <sup>e</sup>	0.04 <sup>cd</sup>	0.004 <sup>b</sup>
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	44.00 (41.53) <sup>e</sup>	17.30 <sup>a</sup>	0.88 <sup>c</sup>	2.32 <sup>f</sup>	9.00 <sup>g</sup>	12.80 <sup>e</sup>	31.40 <sup>g</sup>	167.20 <sup>h</sup>	0.02 <sup>d</sup>	0.003 <sup>b</sup>
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	51.00 (45.56) <sup>d</sup>	17.00 <sup>a</sup>	0.93 <sup>c</sup>	2.95 <sup>e</sup>	9.40 <sup>fg</sup>	12.60 <sup>e</sup>	38.20 <sup>f</sup>	185.80 <sup>g</sup>	0.03 <sup>cd</sup>	0.004 <sup>b</sup>
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	56.00 (48.43) <sup>d</sup>	16.00 <sup>c</sup>	1.01 <sup>c</sup>	3.50 <sup>d</sup>	10.00 <sup>ef</sup>	14.20 <sup>d</sup>	41.60 <sup>ef</sup>	191.60 <sup>f</sup>	0.03 <sup>cd</sup>	0.004 <sup>b</sup>
T <sub>0</sub> : Control	92.00 (74.01) <sup>a</sup>	13.90 <sup>e</sup>	1.93 <sup>a</sup>	7.48 <sup>a</sup>	13.80 <sup>a</sup>	18.00 <sup>a</sup>	79.20 <sup>a</sup>	285.40 <sup>a</sup>	0.07 <sup>a</sup>	0.010 <sup>b</sup>
SE <sub>(m)</sub> ±	1.213	0.156	0.161	0.128	0.252	0.346	1.406	0.519	0.006	0.001
C.D.(0.05)	3.49	0.45	0.46	0.37	0.72	0.99	4.03	1.49	0.018	0.003

\*Mean of five replications, \*\* Mean of three replications, Figures in the parentheses are angular transformed values

Means followed by the same letter are not significantly different from each other

Similarly with reference to growth index, T<sub>6</sub> had the lowest value of 2.32 which was completely different from rest of the treatments. The treatment T<sub>7</sub> had the value of 2.95 which was at par with T<sub>5</sub> (3.33). Highest growth index was witnessed in T<sub>9</sub> (7.48) which was a very high value.

The data on longevity of adults revealed that the male life span was 9.00 days in T<sub>6</sub> which was at par with only T<sub>7</sub> (9.40 days). The treatment T<sub>7</sub> was at par with T<sub>8</sub> (10.00 days) and T<sub>8</sub> was at par with T<sub>5</sub> (10.20 days). The treatments T<sub>1</sub> to T<sub>4</sub> resulted in significantly higher duration of males (11.00 to 12.40 days), whereas, in control the life span of male was observed to be highest (13.80 days) which was significantly different from rest of the treatments. Thus, it was clear that the adult male life span was reduced in all the zinc treatments as compared to control in which the life span was more.

Similarly, T<sub>7</sub> caused lowest female duration (12.60 days) which was at par with T<sub>6</sub> (12.80 days). The other treatments favoured a little longer life span of females i.e. T<sub>5</sub> caused 14.00 days duration remaining at par with T<sub>8</sub> (14.20 days) while T<sub>3</sub> with 14.80 days duration was on a par with T<sub>4</sub> and T<sub>2</sub>. It was also evident from the study that the control treatment recorded the highest value for female duration (18.00 days) which was significantly very high than other comparable treatments.

A perusal of data depicted in Table 18 revealed that the population of WBPH that was built after a constant time interval on rice plants subjected to various zinc application doses, varied significantly within the treatments. The lowest population of WBPH (31.40) was visualized in T<sub>6</sub> which was statistically different from all the treatments. Closely following T<sub>6</sub>, the treatment T<sub>7</sub> supported 38.20 insects and T<sub>8</sub> inhabited 41.60 insects. There was variation between T<sub>1</sub> and T<sub>2</sub> but T<sub>2</sub> did not differ from T<sub>3</sub> and T<sub>4</sub> treatments. However, the control treatment resulted in 79.20 insects which was significantly very high than all other zinc treatments.

Feeding is a measure of antibiosis, as less feeding can cause less honey dew excretion and less feeding is the resultant effect of strong antibiosis factor. It was evident from the data that least area of honey dew excretion (167.20 mm<sup>2</sup>) was reported from T<sub>6</sub>. The treatment T<sub>7</sub> produced slightly more honey dew (185.80 mm<sup>2</sup>), while T<sub>8</sub> showed an area of 191.60 mm<sup>2</sup>. All the zinc treatments were statistically different from each other and also from the control (T<sub>9</sub>) in which the area of honey dew excretion was high (285.40 mm<sup>2</sup>).

The data on weight gain in both male and female WBPH after feeding on treated plants revealed that though the numerical value for weight gain in various treatments seems to be very small, yet, significant variation has been observed among the treatments. The body weight gain for female was minimum (0.02 mg) in T<sub>6</sub> which was quite different from rest of the treatments. The other treatments *viz.*, T<sub>7</sub>, T<sub>4</sub> and T<sub>8</sub> favoured an equal amount of body gain. The highest body weight gain (0.07 mg) was seen in control (T<sub>9</sub>).

The body weight gain in male was found to be very less; nearly 10 times less than the females. There was no variation statistically in the body weight gain in various treatments, but in control (0.01 mg), the body weight gain was statistically high.

#### **4.4.3 Tolerance mechanism induced by zinc**

##### **4.4.3.1 Influence of zinc application on various tolerance parameters of WBPH in rice**

The data on various tolerance mechanism induced in the rice plants on being subjected to various doses of zinc fertilization has been presented in Table 19. It was evident from the Table 19 that the treatment T<sub>6</sub> took 27.67 days to wilt under high pest load which was at par with T<sub>7</sub> (25.00 days) and T<sub>5</sub> (24.33 days). In other treatments, the duration for wilting of the plants ranged from 21.33 days in T<sub>1</sub> to 23.00 days in T<sub>4</sub>. However, the control plants withered in 17.33 days which was completely different from rest of the treatments.

The functional plant loss index (FPLI) was found to be the least (27.80 %) in T<sub>6</sub> which was at par with most of the treatments except T<sub>1</sub> (34.16 %) and T<sub>2</sub> (33.48 %). The control treatment had the highest value of FPLI (69.51 %) which was significantly different from rest of the treatments.

The results of the study on loss in plant biomass as a result of feeding by WBPH have been depicted in Table 19. It can be witnessed from the Table 19 that the loss was minimum in T<sub>7</sub> (59.44 %) which was statistically at par with most of the treatments excluding T<sub>1</sub> and T<sub>2</sub> in which the loss was 65.31 and 64.59 per cent, respectively. Maximum plant biomass loss was observed in T<sub>9</sub> (70.43 %) which was significantly different from all the zinc applied treatments except T<sub>1</sub>. It was further visualized that the grain weight loss was minimum in T<sub>7</sub> (51.95 %) which was at par with T<sub>6</sub> (53.26 %) and T<sub>8</sub> (54.74 %). The control treatment accounted for 78.49 % grain weight loss which was significantly very high.

**Table 19. Influence of zinc application on various tolerance parameters of WBPH in rice**

Treatment	Days to wilt*	FPLI (%)*	Loss in plant biomass (%)	
			Straw weight*	Grain weight*
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	21.33 <sup>bc</sup>	34.16 ( 35.75) <sup>b</sup>	65.31 <sup>ab</sup>	66.01 <sup>b</sup>
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	22.00 <sup>b</sup>	33.48 ( 35.34) <sup>b</sup>	64.59 <sup>bc</sup>	65.27 <sup>b</sup>
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	22.67 <sup>b</sup>	31.29 (33.98) <sup>bc</sup>	63.72 <sup>bc</sup>	62.99 <sup>bc</sup>
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	23.00 <sup>b</sup>	31.37 (34.05) <sup>bc</sup>	64.21 <sup>bc</sup>	61.70 <sup>bc</sup>
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	24.33 <sup>ab</sup>	28.21 (32.07) <sup>c</sup>	63.31 <sup>bc</sup>	58.56 <sup>cd</sup>
T <sub>6</sub> : Zn EDTA basal (40 kg/ha) + Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	27.67 <sup>a</sup>	27.80 (31.80) <sup>c</sup>	61.15 <sup>bc</sup>	53.26 <sup>de</sup>
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha) + Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	25.00 <sup>ab</sup>	29.73 (33.03) <sup>bc</sup>	59.44 <sup>c</sup>	51.95 <sup>e</sup>
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	22.67 <sup>b</sup>	28.49 (32.24) <sup>bc</sup>	62.23 <sup>bc</sup>	54.74 <sup>de</sup>
T <sub>9</sub> : Control	17.33 <sup>c</sup>	69.51 (56.57) <sup>a</sup>	70.43 <sup>a</sup>	78.49 <sup>a</sup>
SE <sub>(m)</sub> ±	1.358	1.031	1.740	2.006
C.D.(0.05)	4.07	3.09	5.17	5.96

\*Mean of three replications

Figures in the parentheses are angular transformed values

Means followed by the same letter are not significantly different from each other

## 4.5 Biochemical analysis and enzymatic study

### 4.5.1 Impact of zinc on various biochemical parameters of rice

The data on total chlorophyll, total phenol, free amino acid, total soluble protein, total soluble sugar and proline content influenced by zinc application has been presented in Table 20(a). A perusal of data indicated that the chlorophyll content was  $1.85 \text{ mg g}^{-1}$  which was significantly highest ( $T_7$ ) followed by  $T_6$  ( $1.77 \text{ mg g}^{-1}$ ) and  $T_8$  ( $1.73 \text{ mg g}^{-1}$ ). In other treatments, the chlorophyll content ranged between  $1.69 \text{ mg g}^{-1}$  in  $T_5$  to  $1.52 \text{ mg g}^{-1}$  in  $T_2$ , whereas, the control treatment registered the lowest content ( $1.04 \text{ mg g}^{-1}$ ). It was further appraised that the treatment  $T_7$  resulted in 77.88 per cent increase in chlorophyll content as compared to the control treatment. With respect to total phenol content, it was evident that the phenol content was highest in  $T_6$  ( $0.31 \text{ mg g}^{-1}$ ) which was significantly higher than other treatments. The treatments  $T_7$  and  $T_8$  had each of  $0.29 \text{ mg g}^{-1}$  of phenol followed by  $T_5$  ( $0.27 \text{ mg g}^{-1}$ ) and  $T_4$  ( $0.23 \text{ mg g}^{-1}$ ). All the rice plants with zinc treatments had high phenol content as compared to plants in control. However, the increase in phenol content was 181.82 per cent in  $T_6$  when compared against the control. The free amino acid content was lowest in  $T_6$  ( $1.39 \text{ mg g}^{-1}$ ) which was at par with all the treatments except control in which the free amino acid content was the highest ( $1.54 \text{ mg g}^{-1}$ ).

It was revealed from the Table 20(b) that, the total soluble protein content was highest in  $T_6$  ( $10.51 \text{ mg g}^{-1}$ ) which was at par with  $T_8$  ( $10.43 \text{ mg g}^{-1}$ ),  $T_7$  ( $9.93 \text{ mg g}^{-1}$ ),  $T_5$  ( $9.67 \text{ mg g}^{-1}$ ) and  $T_4$  ( $9.62 \text{ mg g}^{-1}$ ). The control treatment has  $8.76 \text{ mg g}^{-1}$  of total soluble protein which was at par with  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$  treatments. Thus, from the Table 20(b) it was inferred that  $T_6$ ,  $T_7$  and  $T_8$  treatments significantly increased total soluble protein content in rice plants. The quantity of total soluble sugar was found to be lowest in  $T_6$  ( $61.91 \text{ mg g}^{-1}$ ) which was at par with many treatments except for  $T_1$  ( $69.22 \text{ mg g}^{-1}$ ), whereas, in control treatment, the same value was  $72.08 \text{ mg g}^{-1}$  which was at par with  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$  treatments. The treatments  $T_5$ ,  $T_6$ ,  $T_7$  and  $T_8$  contained 10.03 to 14.11 per cent less total soluble sugar as compared to control. As regards to proline content, it was ascertained that proline content was lowest in  $T_6$  ( $7.12 \text{ } \mu\text{g g}^{-1}$ ) which was at par with  $T_7$  and  $T_8$  treatments. The treatment  $T_5$  contained  $7.85 \text{ } \mu\text{g g}^{-1}$  which was at par with rest of the treatments including control which clearly depicted that,  $T_6$ ,  $T_7$  and  $T_8$  treatments effectively reduced the biotic stress for which there was a decline in the proline content.

**Table 20(a). Effect of zinc application on biochemical parameters of rice plants**

Treatment	Total Chlorophyll (mg g <sup>-1</sup> )	Increase over control (%)	Total Phenol (mg g <sup>-1</sup> )	Increase over control (%)	Free amino acid (mg g <sup>-1</sup> )	Decrease over control (%)
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	1.55 <sup>c</sup>	49.04	0.19 <sup>cd</sup>	72.73	1.52 <sup>ab</sup>	1.30
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	1.52 <sup>c</sup>	46.15	0.15 <sup>de</sup>	36.37	1.51 <sup>ab</sup>	1.95
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	1.62 <sup>bc</sup>	55.78	0.21 <sup>c</sup>	90.91	1.51 <sup>ab</sup>	1.95
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	1.67 <sup>bc</sup>	60.58	0.23 <sup>bc</sup>	109.09	1.50 <sup>abc</sup>	2.60
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	1.69 <sup>ab</sup>	62.50	0.27 <sup>ab</sup>	145.45	1.48 <sup>abc</sup>	3.90
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	1.77 <sup>ab</sup>	70.19	0.31 <sup>a</sup>	181.82	1.39 <sup>c</sup>	9.74
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha) + Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	1.85 <sup>a</sup>	77.88	0.29 <sup>a</sup>	163.64	1.44 <sup>abc</sup>	6.49
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	1.73 <sup>ab</sup>	66.35	0.29 <sup>a</sup>	163.64	1.41 <sup>bc</sup>	8.44
T <sub>9</sub> : Control	1.04 <sup>d</sup>	-	0.11 <sup>e</sup>	-	1.54 <sup>a</sup>	-
SE <sub>(m)</sub> ±	0.058		0.012		0.036	
C.D.(0.05)	0.17		0.04		0.11	

Means followed by the same letter are not significantly different from each other

**Table 20(b). Effect of zinc application on biochemical parameters of rice plants**

Treatment	Total soluble protein (mg g <sup>-1</sup> )	Increase over control (%)	Total soluble sugar (mg g <sup>-1</sup> )	Decrease over control (%)	Proline (µg g <sup>-1</sup> )	Decrease over control (%)
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	8.89 <sup>c</sup>	1.48	69.22 <sup>ab</sup>	3.98	8.27 <sup>a</sup>	0.84
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	9.15 <sup>bc</sup>	4.45	67.47 <sup>abc</sup>	6.40	8.19 <sup>ab</sup>	1.80
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	9.31 <sup>bc</sup>	6.28	65.95 <sup>abc</sup>	8.50	7.99 <sup>abc</sup>	4.20
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	9.62 <sup>abc</sup>	9.82	66.39 <sup>abc</sup>	7.89	8.07 <sup>ab</sup>	3.24
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	9.67 <sup>abc</sup>	10.39	64.85 <sup>abc</sup>	10.03	7.85 <sup>abc</sup>	5.88
T <sub>6</sub> : Zn EDTA basal (40 kg/ha) + Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	10.51 <sup>a</sup>	19.98	61.91 <sup>c</sup>	14.11	7.12 <sup>d</sup>	14.63
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha) + Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	9.93 <sup>ab</sup>	13.36	62.33 <sup>c</sup>	13.53	7.63 <sup>bcd</sup>	8.51
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	10.43 <sup>a</sup>	19.06	62.94 <sup>bc</sup>	12.68	7.61 <sup>cd</sup>	8.75
T <sub>9</sub> : Control	8.76 <sup>c</sup>	-	72.08 <sup>a</sup>	-	8.34 <sup>a</sup>	-
SE <sub>(m)</sub> ±	0.327		2.113		0.194	
C.D.(0.05)	0.97		6.28		0.57	

Means followed by the same letter are not significantly different from each other

#### **4.5.2 Impact of zinc on enzymatic activity of rice**

The data on activity of super oxide dismutase (SOD), peroxidase (POD) and Phenylalanine ammonia lyase (PAL) influenced by zinc application has been depicted in Table 21. It was revealed from the Table 21 that SOD activity was observed to be maximum in T<sub>6</sub> (4.21 Units min<sup>-1</sup> mg<sup>-1</sup> protein) which was at par with T<sub>5</sub>, T<sub>7</sub> and T<sub>8</sub> treatments. The treatments T<sub>7</sub> and T<sub>8</sub> remained at par with each other. The rest of the treatments remained at par among themselves but the control treatment (3.90 Units min<sup>-1</sup> mg<sup>-1</sup> protein) was significantly different from all other treatments. As regards to POD activity, T<sub>6</sub> had the highest value (4.08 Units min<sup>-1</sup> mg<sup>-1</sup> protein) which was at par with T<sub>7</sub> and T<sub>5</sub> treatments. Rest other treatments were at par within themselves but all the treatments were superior to control (3.81 Units min<sup>-1</sup> mg<sup>-1</sup> protein). PAL activity was highest in T<sub>6</sub> (7.15 Units hr<sup>-1</sup> mg<sup>-1</sup> protein) which was at par with all the treatments except T<sub>1</sub>, whereas, the control treatment possessed lowest PAL activity (6.80 Units hr<sup>-1</sup> mg<sup>-1</sup> protein).

#### **4.6 Impact of zinc application on zinc content of rice plant during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (field experiment)**

It was observed from Table 22 that zinc content was maximum in T<sub>6</sub> (23.60, 22.40 ppm) during *Kharif*, 2016 and summer, 2016-17, whereas, in *kharif*, 2017, the treatment T<sub>5</sub> had the highest zinc content (22.20 ppm). Irrespective of the season tested, the control treatment had significantly lowest zinc content as compared to various treatments. As regards to the pooled mean, it was evident that T<sub>6</sub> had the highest zinc content (22.60 ppm) which was at par with T<sub>7</sub> (21.83 ppm).

#### **4.7 Effect of zinc application on nutrient content (potash and zinc) in pot culture experiment**

The data on potassium and zinc content of rice plants taken from pot culture experiment at 60 DAT has been presented in Table 23. It can be visualized from the data that potassium content was maximum in T<sub>7</sub> (1.41 %) which was at par with T<sub>8</sub> (1.35 %), whereas, T<sub>8</sub> was at par with T<sub>5</sub> (1.29 %) and T<sub>6</sub> (1.28 %). Similarly it was also observed that the potassium content in T<sub>1</sub>, T<sub>3</sub> and T<sub>4</sub> was also at par with T<sub>6</sub>. But in all the treatments comprising zinc supplementation, the potassium content was significantly higher than the control treatment (1.09 %).

**Table 21. Influence of zinc application on enzymatic activity of rice plants**

Treatment	SOD (Units/ min/ mg protein)	Increase over control (%)	POD (Units/ min/ mg protein)	Decrease over control (%)	PAL (Units/ hr/ mg protein)	Increase over control (%)
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	4.06 <sup>bc</sup>	4.10	3.92 <sup>b</sup>	2.89	6.95 <sup>bc</sup>	2.21
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	4.02 <sup>c</sup>	3.07	3.93 <sup>b</sup>	3.15	7.01 <sup>abc</sup>	3.09
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	4.08 <sup>bc</sup>	4.62	3.96 <sup>b</sup>	3.94	7.03 <sup>abc</sup>	3.38
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	4.09 <sup>bc</sup>	4.87	3.95 <sup>b</sup>	3.67	7.05 <sup>abc</sup>	3.68
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	4.11 <sup>abc</sup>	5.38	4.00 <sup>ab</sup>	4.99	7.08 <sup>ab</sup>	4.12
T <sub>6</sub> : Zn EDTA basal (40 kg/ha) + Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	4.21 <sup>a</sup>	7.95	4.08 <sup>a</sup>	7.09	7.15 <sup>a</sup>	5.15
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha) + Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	4.16 <sup>ab</sup>	6.67	4.01 <sup>ab</sup>	5.25	7.09 <sup>ab</sup>	4.26
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	4.14 <sup>ab</sup>	6.15	3.97 <sup>b</sup>	4.20	7.11 <sup>a</sup>	4.56
T <sub>9</sub> : Control	3.90 <sup>d</sup>	-	3.81 <sup>c</sup>	-	6.80 <sup>d</sup>	-
SE <sub>(m)</sub> ±	0.038		0.031		0.047	
C.D.(0.05)	0.11		0.09		0.14	

Means followed by the same letter are not significantly different from each other

**Table 22. Zinc content of rice plant during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (field experiment)**

Treatment	Zinc content (ppm)			Pooled mean	Increase over control (%)
	<i>kharif</i> , 2016	summer, 2016-17	<i>kharif</i> , 2017		
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	18.80 <sup>d</sup>	19.60 <sup>bcd</sup>	20.30 <sup>ab</sup>	19.57 <sup>c</sup>	9.76
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	21.30 <sup>bc</sup>	21.30 <sup>ab</sup>	21.40 <sup>a</sup>	21.33 <sup>b</sup>	19.63
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	20.00 <sup>cd</sup>	19.20 <sup>cd</sup>	18.60 <sup>bc</sup>	19.27 <sup>c</sup>	8.08
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	20.20 <sup>cd</sup>	18.90 <sup>cd</sup>	18.80 <sup>bc</sup>	19.30 <sup>c</sup>	8.24
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	21.00 <sup>bc</sup>	20.50 <sup>abc</sup>	22.20 <sup>a</sup>	21.23 <sup>b</sup>	19.07
T <sub>6</sub> : Zn EDTA basal (40 kg/ha) + Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	23.60 <sup>a</sup>	22.40 <sup>a</sup>	21.80 <sup>a</sup>	22.60 <sup>a</sup>	26.75
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha) + Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	22.50 <sup>ab</sup>	21.40 <sup>ab</sup>	21.60 <sup>a</sup>	21.83 <sup>ab</sup>	22.43
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	21.50 <sup>bc</sup>	20.70 <sup>abc</sup>	22.10 <sup>a</sup>	21.43 <sup>b</sup>	6.74
T <sub>9</sub> : Control	18.70 <sup>d</sup>	17.80 <sup>d</sup>	17.00 <sup>c</sup>	17.83 <sup>d</sup>	-
SE <sub>(m)</sub> ±	0.611	0.641	0.670	0.378	-
C.D.(0.05)	1.83	1.92	2.01	1.07	-

Means followed by the same letter are not significantly different from each other

**Table 23. Potassium and zinc content of rice plant during *kharif*, 2017 (Pot culture experiment)**

<b>Treatment</b>	<b>Potassium (%)</b>	<b>Increase over control (%)</b>	<b>Zinc (ppm)</b>	<b>Increase over control (%)</b>
T <sub>1</sub> : ZnSO <sub>4</sub> basal (25kg/ha)	1.21 <sup>cd</sup>	11.01	16.80 <sup>de</sup>	5.00
T <sub>2</sub> : Zn EDTA basal (40 kg/ha)	1.14 <sup>de</sup>	4.59	20.20 <sup>abc</sup>	26.25
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	1.21 <sup>cd</sup>	11.00	18.50 <sup>cd</sup>	15.62
T <sub>4</sub> : Zn EDTA Foliar spray @ 0.8% (8g/l of water) twice at 30 and 45 DAT	1.23 <sup>cd</sup>	12.84	19.10 <sup>bc</sup>	19.37
T <sub>5</sub> : ZnSO <sub>4</sub> basal (25kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	1.29 <sup>bc</sup>	18.35	21.00 <sup>ab</sup>	31.25
T <sub>6</sub> : Zn EDTA basal (40 kg/ha)+ Zn EDTA Foliar spray@ 0.8% (8 g/l of water)twice at 30 and 45 DAT	1.28 <sup>bc</sup>	17.43	21.30 <sup>a</sup>	33.12
T <sub>7</sub> : ZnSO <sub>4</sub> basal(25kg/ha)+ Zn EDTA Foliar spray @ 0.8% (8 g/l of water) twice at 30 and 45 DAT	1.41 <sup>a</sup>	29.36	20.50 <sup>ab</sup>	28.13
T <sub>8</sub> : Zn EDTA basal (40 kg/ha) + ZnSO <sub>4</sub> foliar spray @ 0.5% (5g/l of water) twice at 30 and 45 DAT	1.35 <sup>ab</sup>	23.85	21.30 <sup>a</sup>	33.12
T <sub>9</sub> : Control	1.09 <sup>e</sup>	-	16.00 <sup>e</sup>	-
SE <sub>(m)</sub> ±	0.029		0.643	
C.D.(0.05)	0.09		1.91	

Means followed by the same letter are not significantly different from each other

It can be depicted from Table 23 that zinc content was maximum in T<sub>6</sub> as well as in T<sub>8</sub> i.e. 21.30 ppm, which was at par with T<sub>2</sub> (20.20 ppm), T<sub>7</sub> (20.50 ppm) and T<sub>5</sub> (21.00 ppm). The treatment T<sub>9</sub> (control) had significantly lowest zinc content (16.00 ppm) which was significantly different from all the treatments.

#### **4.8 Correlation between zinc content of the plant samples with plant growth parameters and WBPH population during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (pooled) at 60 DAT**

The correlation study between the zinc content of the plant with various plant growth parameters and WBPH population at highest pest activity was undertaken and the data has been presented in Table 24. It can be visualized from the Table that zinc content was positively correlated with number of tillers regardless of the season under study. Zinc content was also positively related to plant height in all the three seasons. While, the number of panicle/m<sup>2</sup> though did not show any significant correlation with zinc content during *kharif*, 2016, however, it was positively correlated during both summer, 2016-17 and *kharif*, 2017. Similarly, the number of grains per panicle and the grain yield were positively correlated with zinc content. As regards to the pooled 'r' values, it can be visualized that all the plant morphological parameters *viz.*, number of tillers/hill, plant height, number of panicle/m<sup>2</sup>, number of grain/panicle and grain yield were positively correlated to zinc content ('r' values of 0.96, 0.83, 0.91, 0.97 and 0.95, respectively).

The WBPH population at 60 DAT (maximum pest activity) was correlated with zinc content of the plant samples. It can be perused from the Table that WBPH population was significantly and negatively correlated during *kharif*, 2016 ( $r = -0.82$ ), summer, 2016-17 ( $r = -0.78$ ) and *kharif*, 2017 ( $r = -0.69$ ), whereas, the pooled 'r' value (-0.85) was negatively correlated with zinc content.

#### **4.9 Correlation between zinc content of the plant samples with plant biochemical parameters (pot culture) during *kharif*, 2017**

The correlation study between zinc content of the plant and various plant biochemical parameters was undertaken during *kharif*, 2017 and the data has been presented in Table 25. It can be visualized that the zinc content was positively

**Table 24. Correlation between zinc content of the plant samples with plant growth parameters and WBPH population during *kharif*, 2016, summer, 2016-17 and *kharif*, 2017 (pooled) at 60 DAT**

Correlation study parameters	Correlation coefficient (r)			
	<i>kharif</i> , 2016	summer, 2016-17	<i>kharif</i> , 2017	Pooled
Zinc content vs. number of tillers	0.66*	0.92**	0.95**	0.96**
Zinc content vs. plant height	0.66*	0.70*	0.89**	0.83**
Zinc content vs. number of panicle/m <sup>2</sup>	0.64	0.89**	0.97**	0.91**
Zinc content vs. number of grains/ panicle	0.87**	0.94**	0.95**	0.97**
Zinc content vs. grain yield	0.85**	0.90**	0.79*	0.95**
Zinc content vs. WBPH population	-0.82**	-0.78*	-0.69*	-0.85**

\* Significant at 5% level

\*\* Significant at both 1% and 5% level

**Table 25. Correlation between zinc content of the plant samples with plant biochemical parameters during *kharif*, 2017**

Correlation study parameters	Correlation coefficient (r)
Zinc content vs. total chlorophyll	0.73*
Zinc content vs. total phenol	0.78*
Zinc content vs. free amino acid	-0.79*
Zinc content vs. total soluble protein	0.87**
Zinc content vs. total soluble sugar	-0.86**
Zinc content vs. proline content	-0.76*
Zinc content vs. SOD activity	0.72*
Zinc content vs. POD activity	0.76*
Zinc content vs. PAL activity	0.92**

\* Significant at 5% level

\*\* Significant at both 1% and 5% level

correlated with total chlorophyll ( $r = 0.73$ ), total phenol ( $r = 0.78$ ), total soluble protein ( $r = 0.87$ ), SOD activity ( $r = 0.72$ ), POD activity ( $r = 0.76$ ) and PAL activity ( $r = 0.92$ ), whereas, the zinc content was negatively correlated to free amino acid ( $r = -0.79$ ), total soluble sugar ( $r = -0.86$ ) and proline content ( $r = -0.76$ ), respectively.

#### **4.10 Correlation between WBPH population build up with plant biochemical parameters during *kharif*, 2017**

The correlation study between population build up of WBPH and various plant biochemical parameters was also undertaken and the data has been depicted in Table 26. It can be envisaged from the Table that the WBPH population build up was positively correlated to free amino acid ( $r = 0.82$ ), total soluble sugar ( $r = 0.94$ ) and proline content ( $r = 0.82$ ), whereas, it was negatively correlated to total chlorophyll ( $r = -0.96$ ), total phenol ( $r = -0.90$ ), total soluble protein ( $r = -0.83$ ), SOD activity ( $r = -0.88$ ), POD activity ( $r = -0.90$ ) and PAL activity ( $r = -0.91$ ).

#### **4.11 Analysis of protein profile**

Zinc serves as a co-factor for more than 300 enzymes in plant and animal system. Altogether, nineteen polypeptide bands were revealed in response to extraneous application of zinc in form of basal and foliar application of  $ZnSO_4$  and Zn EDTA. Polypeptide bands i.e. B1 (97.4 kDa), B2 (90 kDa), B4 (78 kDa), B8 (40.2 kDa), and B13 (27.3 kDa) in the zymogram are monomorphic over all the treatments and control indicating their expression independent of the zinc application (Table 27 and Figure 14). It was observed that polypeptide bands of 29.0 kDa, 35.0 kDa, 56.8 kDa and 85.5 kDa were absent in basal application of  $ZnSO_4$ , but induced in all treatments and even in control. In contrast, 33.0 kDa and 25.1 kDa polypeptides were induced by basal application of  $ZnSO_4$ , but down-regulated in all treatments including control. Zinc uptake in case of basal application is determined by presence of Zn-transporter genes in root cells followed by transport to stem and foliage, while foliar application can bypass such genetic system and avail zinc directly to the enzymes required for plant growth and metabolism. Basal application of Zn EDTA and also foliar application of  $ZnSO_4$  alone produced a polypeptide band of 20.1 kDa. Two polypeptide bands *viz.*, 37.0 kDa and 66 kDa were induced in  $ZnSO_4$  basal, but not expressed in control as well as in response to its foliar spray alone or its additional application as foliar spray.

**Table 26. Correlation between population build up of WBPH with various plant biochemical parameters during *kharif*, 2017**

<b>Correlation study parameters</b>	<b>Correlation coefficient (r)</b>
Population build up vs. total chlorophyll	-0.96**
Population build up vs. total phenol	-0.90**
Population build up vs. free amino acid	0.82**
Population build up vs. total soluble protein	-0.83**
Population build up vs. total soluble sugar	0.94**
Population build up vs. proline content	0.82**
Population build up vs. SOD activity	-0.88**
Population build up vs. POD activity	-0.90**
Population build up vs. PAL activity	-0.91**

\*\* Significant at both 1% and 5% level

**Table 27. SDS-PAGE Polypeptide banding pattern of total soluble protein samples extracted from rice leaves after application of zinc**

<b>Poly-peptide band</b>	<b>Mol. Wt. (kDa)</b>	<b>T<sub>1</sub> ZnSO<sub>4</sub> basal (25kg/ha)</b>	<b>T<sub>2</sub> Zn EDTA basal (40kg/ha)</b>	<b>T<sub>3</sub> ZnSO<sub>4</sub> foliar spray (0.5%) (30 and 45 DAT)</b>	<b>T<sub>4</sub> Zn EDTA foliar spray (0.8%) (30 and 45 DAT)</b>	<b>T<sub>5</sub> T<sub>1</sub> + T<sub>3</sub></b>	<b>T<sub>6</sub> T<sub>2</sub> + T<sub>4</sub></b>	<b>T<sub>7</sub> T<sub>1</sub> + T<sub>4</sub></b>	<b>T<sub>8</sub> T<sub>2</sub> + T<sub>3</sub></b>	<b>T<sub>9</sub> Control</b>
B1	97.4	1	1	1	1	1	1	1	1	1
B2	90.0	1	1	1	1	1	1	1	1	1
B3	85.5	0	1	1	1	1	1	1	1	1
B4	78.0	1	1	1	1	1	1	1	1	1
B5	72.2	1	1	1	1	1	1	0	1	1
B6	66.0	1	0	0	0	0	1	1	1	0
B7	56.8	0	1	1	1	1	1	1	1	1
B8	40.2	1	1	1	1	1	1	1	1	1
B9	37.0	1	0	0	0	0	1	1	1	0
B10	35.0	0	1	1	1	1	1	1	1	1
B11	33.0	1	0	0	0	0	0	0	0	0
B12	29.0	0	1	1	1	1	1	1	1	1
B13	27.3	1	1	1	1	1	1	1	1	1
B14	25.1	1	0	0	0	0	0	0	0	0
B15	23.6	0	0	0	0	0	1	1	0	0
B16	22.0	0	1	1	1	1	0	1	0	0
B17	20.1	0	1	1	0	0	0	0	0	0
B18	15.8	0	0	0	1	1	1	0	1	0
B19	14.3	0	1	1	1	1	1	1	1	0
Total bands		10	13	13	13	13	15	14	14	10
WBPH population build up		53.60	51.20	49.80	49.00	45.20	31.40	38.20	41.60	79.20

1: Present, 0: Absent

Even these two polypeptide bands were also not expressed in Zn EDTA basal or foliar spray alone, but induced by combination of basal + foliar spray of Zn EDTA as well as combination of basal ZnSO<sub>4</sub> + foliar Zn EDTA or vice versa. The low molecular weight proteins ranging from 14.3-25.1 kDa were clearly absent in the control, but zinc application in form of above sources as basal or foliar treatment elicited biosynthesis of new polypeptide bands. For instance, a polypeptide band (14.3 kDa) was noticed in all the zinc treatments except ZnSO<sub>4</sub> basal. Besides, foliar spray of Zn EDTA in combination with basal application of either ZnSO<sub>4</sub> or Zn EDTA induced new polypeptide band at 23.6 kDa. In the present investigation, elucidation of the induced polypeptide bands revealed in response to various treatments of zinc-application may throw light for better understanding about the biochemical basis of induced host plant resistance.

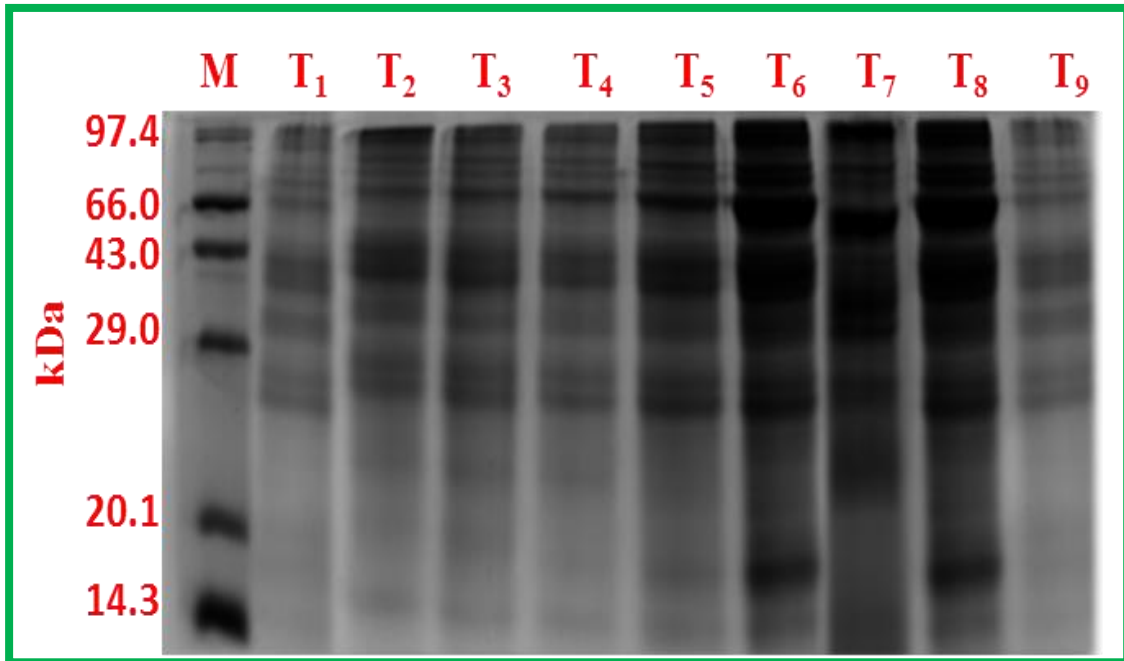
WBPH is a dreadful sucking insect of rice and it usually attack at culm base of rice plant at tillering and flowering stage causing drastic reduction in crop growth and seed yield. Zinc application either as basal and/or foliar application reduced the population build up of WBPH ranging from 31.40 to 53.60 insects/hill as against control (79.20 insects/hill). Combination treatment of basal and foliar application of zinc formulation in the form of Zn EDTA (T<sub>6</sub>: T<sub>2</sub> + T<sub>4</sub>) resulted maximum dividend followed by T<sub>7</sub> (T<sub>1</sub> + T<sub>4</sub>) and T<sub>8</sub> (T<sub>2</sub> + T<sub>3</sub>) in term of decrease in WBPH population. It is worth to note that the most responsive zinc combination treatment (T<sub>6</sub>) elicited highest number (15) of polypeptide bands against ten normal protein bands in the control. Expression of five new polypeptide bands at 66.0, 37.0, 23.6, 15.8 and 14.3 kDa induced by T<sub>6</sub> can be considered as biochemical basis of induced resistance in rice against WBPH. Among these, 66.0, 37.0, 23.6 and 14.3 kDa protein bands were induced by T<sub>7</sub> and 66.0, 37.0, 15.8 and 14.3 kDa protein bands induced by T<sub>8</sub>, were common to that of T<sub>6</sub>, which were new types and not expressed in control treatment. Further, the soluble protein profiling study revealed that 66.0 kDa, 37.0 kDa and 14.3 kDa polypeptide bands were significantly induced and commonly shared in T<sub>6</sub>, T<sub>7</sub> and T<sub>8</sub> that recorded lower WBPH population. However, 23.6 kDa polypeptide band induced only in T<sub>6</sub> and T<sub>7</sub> seems to have greater role in manifestation of induced resistance to WBPH in rice.

Further, clustering pattern/ dendrogram (Figure 15) based on pair-wise similarity coefficient values (Table 28) revealed that T<sub>1</sub>, T<sub>9</sub> (Control); T<sub>6</sub>, T<sub>7</sub> and T<sub>8</sub>

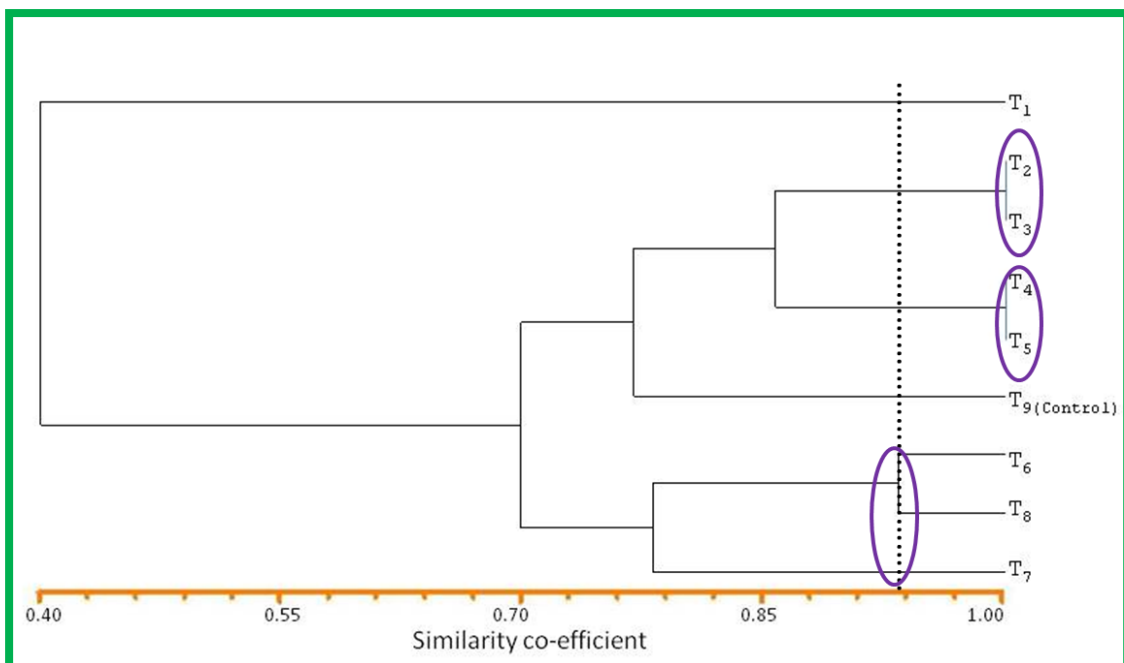
formed single treatment cluster while, both T<sub>2</sub> and T<sub>3</sub> together and also T<sub>4</sub> and T<sub>5</sub> combinedly formed separate clusters at 100 per cent phenon level. Had there been fine tuning of the protein profiling T<sub>2</sub> and T<sub>3</sub> might be separated and this difference could have been interpreted in terms of per cent damage difference between T<sub>2</sub> and T<sub>3</sub>. Similarly, T<sub>4</sub> and T<sub>5</sub> could have been differentiated as we have got difference between these in field and pot culture experiment. However, the difference between T<sub>4</sub> and T<sub>5</sub> was not so much vigilant as compared to their effect with either of T<sub>6</sub>, T<sub>7</sub> or T<sub>8</sub>, which revealed higher induction of host plant resistance to WBPH.

**Table 28. Similarity coefficient between zinc treatments for induction of resistance to white backed plant hopper in rice**

<b>Treatment</b>	<b>T<sub>1</sub> ZnSO<sub>4</sub> basal (25kg/ha)</b>	<b>T<sub>2</sub> Zn EDTA basal (40kg/ha)</b>	<b>T<sub>3</sub> ZnSO<sub>4</sub> foliar spray (0.5%) (30 and 45 DAT)</b>	<b>T<sub>4</sub> Zn EDTA foliar spray (0.8%) (30 and 45 DAT)</b>	<b>T<sub>5</sub> T<sub>1</sub> + T<sub>3</sub></b>	<b>T<sub>6</sub> T<sub>2</sub> + T<sub>4</sub></b>	<b>T<sub>7</sub> T<sub>1</sub> + T<sub>4</sub></b>	<b>T<sub>8</sub> T<sub>2</sub> + T<sub>3</sub></b>	<b>T<sub>9</sub> Control</b>
T <sub>2</sub> : Zn EDTA basal (40kg/ha)	0.35								
T <sub>3</sub> : ZnSO <sub>4</sub> foliar spray (0.5%) (30 and 45 DAT)	0.35	1.00							
T <sub>4</sub> : Zn EDTA foliar spray (0.8%) (30 and 45 DAT)	0.35	0.86	0.86						
T <sub>5</sub> : T <sub>1</sub> + T <sub>3</sub>	0.35	0.86	0.86	1.00					
T <sub>6</sub> : T <sub>2</sub> + T <sub>4</sub>	0.47	0.65	0.65	0.75	0.75				
T <sub>7</sub> : T <sub>1</sub> + T <sub>4</sub>	0.41	0.69	0.69	0.69	0.69	0.81			
T <sub>8</sub> : T <sub>2</sub> + T <sub>3</sub>	0.50	0.69	0.69	0.80	0.80	0.93	0.75		
T <sub>9</sub> : Control	0.43	0.77	0.77	0.77	0.77	0.67	0.60	0.71	



**Fig.14: Impact of zinc application on gel banding pattern of total soluble protein profile of rice leaf by SDS-PAGE method**



**Fig. 15: Dendrogram depicting variability among various treatments**

## DISCUSSION

The study on “Induction of resistance in rice to white backed plant hopper, *Sogatella furcifera* (Horvath) through application of zinc” was undertaken in field, pot culture and laboratory conditions and the data pertaining to each experiment has been depicted in previous chapter. The findings of the various experiments are critically discussed in this section.

### 5.1 Incidence of white backed plant hopper (WBPH) under field condition

Regardless of the seasons under study, it was evidenced that in control treatment, highest WBPH population was noticed in rice at about 60 days after transplanting. It was further observed that the population during *kharif* season was more than that observed in summer season. During *kharif*, 2016, summer, 2016-17 and *kharif*, 2017; the highest WBPH population per hill was 44.66, 14.13 and 24.18, respectively in control treatment (Table 4 to 6). In all the zinc treatments, there was a marked decline in WBPH population irrespective of the seasons studied. It has been also witnessed that higher WBPH population on rice coincided with maximum tillering stage (55-60 DAT) of crop (CRIDA, 2019). The flare up of WBPH population requires an ideal ecological conditions i.e. a temperature range of more than 28<sup>0</sup>C with a corresponding relative humidity (RH) of around 80 per cent or more (Win *et al.*, 2011). In our finding, low number of WBPH during summer, 2016-17 than *kharif* seasons may be attributable to unfavourable RH factor.

Application of zinc in rice substantially reduced the WBPH number per hill when compared to the control (Table 7). The pooled data revealed that application of Zn EDTA (basal) with foliar spray of Zn EDTA (twice @ 0.8 %) (T<sub>6</sub>) caused an overall 66.15 per cent reduction in WBPH population when it was compared with the control. The next better treatment was T<sub>7</sub> which also accounted for 60.84 per cent reduction in WBPH population. Thus, it was clearly evident that zinc might have definitely influenced rice plant physiology which in turn also influenced growth and development of WBPH. Reduction in WBPH population due to zinc application in rice also has been observed by Rath and Mishra (1998), Rath (2004) and Dash and Mishra (2009).

## 5.2 Effect of zinc on various growth attributes of rice plant

Various growth parameters of rice *viz.*, tiller numbers/hill, (Table 8 to 11), plant height (Table 12), number of panicles/m<sup>2</sup> (Table 13) and number of grains/ panicle (Table 14) under the influence of zinc was studied under field condition. Application of zinc increased the tiller number in all the test seasons as compared to no application (control). It was further observed that the tiller number was more than double in T<sub>5</sub> during *kharif*, 2016; nearing double in T<sub>6</sub> both in summer, 2016-17 and *kharif*, 2017, respectively. The pooled data also revealed that the treatments T<sub>7</sub>, T<sub>6</sub> and T<sub>5</sub> produced 70.71, 69.59 and 68.90 per cent more tillers, respectively as against control (Table 11). Increase in tiller number due to zinc application was attributable to improved enzymatic activity and auxin metabolism (Khan *et al.*, 2007). Ghoneim (2016) discussed that the increase in tiller number due to zinc application was due to improved availability of nutrients to the plants from soil. Increase in tiller number is definitely a boost in growth of rice hill and it is definitely the growth regulators like auxin, gibberellin etc. which accelerate the production of additional tillers. In the present study, we observed a similar phenomenon as observed by the earlier workers.

Application of zinc in rice also caused significant increase in plant height when compared against the control treatment (Table 12). It was quite interesting to observe that there was no variation within the zinc treatments regardless of the seasons under study, but in all the cases addition of zinc enhanced the plant height than the control treatment. Hence, zinc exercised a probable phytotonic effect. Sudha and Stalin (2015) have described such a boosting in plant height in rice as an 'auxin boost'. Kumari *et al.* (2019) also have opined that zinc increased the auxin level in rice plants which was responsible for elongation of coleoptile and stem and as a result, there was ultimate increase in plant height. Muamba and Ambara (2013) also witnessed a similar phenomenon and thus, the present finding lie in the same line of the finding of the above authors.

As regards to number of panicle/m<sup>2</sup>, it was observed that there was substantial increase in panicle number in zinc treated plots as against the control plot. Even within the zinc treatments, variation did exist. It was also evident that sole basal application of zinc was more potential than sole foliar spray. Thus, it was clear that basal application of zinc favoured more number of tillers per hill for which the panicle number also increased than the only foliar spray treatments. But rest other treatments having a blend of both basal and foliar spray produced more number of panicles, though remained

statistically at par with only basal application treatments. A focus on pooled mean that clearly demonstrated increased tiller number in all the treatments of zinc than control and the increase was 17.19 to 70.90 per cent (Table 13). Ghasal *et al.* (2015) also observed highest number of panicle/m<sup>2</sup> (350) in aromatic rice, Pusa Rice Hybrid 10, with application of 1.25 kg Zn/ha (Zn-EDTA) + 0.5 % foliar spray at maximum tillering and panicle initiation stage. The present finding corroborate with the above finding.

Grain number per panicle in rice is an important yield attributing character than long panicles with less grains. Along with grain number, grain boldness also accounts for higher yield output. Over the seasons, it was observed that zinc applied plots recorded higher number of grains per panicle than the control treatment (Table 14). The treatments T<sub>6</sub>, T<sub>7</sub> and T<sub>5</sub> produced comparatively higher number of grains per panicle than corresponding other zinc treatments and with regards to control treatment, the above three treatments recorded 23.53, 22.01 and 20.28 per cent more grains per panicle. Higher number of grains per panicle has also been documented by Sudha and Stalin (2015), who described that more grains per panicle was due to increase in panicle length to accommodate more grains. Shivay *et al.* (2010) also studied higher grain number per panicle due to application of zinc. Thus, the present finding derived ample support from the above findings.

The grain yield is a function of tiller number, effective tiller number, number of grains per panicle, panicle length and thousand seed weight etc. The grain yield in all the test seasons was found to be high in T<sub>7</sub> (37.27 q/ha) during *kharif*, 2016, in T<sub>6</sub> (36.50 q/ha) during summer, 2016-17 and again in T<sub>6</sub> (31.35 q/ha) during *kharif*, 2017 (Table 15). Though within the zinc treatments, variation existed but invariably all the zinc applications were superior than control treatment. It was also visualized that zinc application caused as low as 16.54 to as high as 74.17 per cent increase in grain yield over control. Mumba and Ambara (2013) have observed that zinc application improved the photosynthesis rate in rice through chlorophyll formation. Mousavi (2011) also reported that either basal or foliar application of zinc increased the biosynthesis of chlorophyll which was the key process for higher grain yield. Similar study also has been made by Mathpal *et al.* (2015). Thus, the grain yield which is dependent upon number of tillers, number of grains per panicle were enhanced in the present study and the higher grain yield that was witnessed in zinc treatment was also observed by above workers.

### 5.3 Induced effect of zinc for antixenosis against WBPH

Antixenosis mechanism of resistance expression in plants may be either constitutive or induced. Antixenosis mechanism in terms of nymphal alightment, adult WBPH alightment, rate of oviposition and per cent egg that did not hatch were studied and the result has been presented in Table 16 and 17. It was evident from nymphal alightment study that more percentage of WBPH nymphs were attracted to the rice plants devoid of zinc treatment (control), whereas, zinc treated plants attracted approximately half the nymphs as compared to control. Similarly, the adult insects were more attracted to control plants than the treated plants in a free choice test. Reduced attraction to zinc treated plants by both WBPH nymphs and adults could be attributable to first olfactory response to plant volatiles and second to gustatory stimuli. Since, at the end of 72 hours of post-release period, the per cent orientation increased on control plants, it can be inferred that the insects might have discriminated the plants after preliminary gustatory response. But the role of different blends of plant volatiles emanating from the plants to exercise olfactory stimuli in insects for orientation cannot be completely ruled out. Whether the antixenosis through host attraction was due to gustatory response or olfactory response, irrespective of the modality it was witnessed that nymphal and adult attraction was marked more in control than on zinc treated rice plants. Pathak and Saxena (1980), Sogawa and Pathak (1970) and Gunathilagraj and Chelliah (1985) have opined that the orientation of hoppers (plant hoppers of rice) on susceptible and resistance rice lines was due to gustatory response. Rath (1995) have reported less nymphal and adult orientation of WBPH on zinc treated rice plants than untreated TN 1 plants. The present finding is in conformity with findings of the above authors.

It was further witnessed that the WBPH female adults laid significantly less number of eggs on rice plants treated with zinc as against the plants without zinc application (Table 17). The treatment T<sub>6</sub> only recorded 34.40 eggs as against 86.2 eggs in control. Thus, nearly 60 per cent less eggs were laid in T<sub>6</sub> when compared with control. Invariably all the zinc treatment supported significantly less eggs than control. Therefore, it was indicated that application of zinc and ultimate uptake of zinc definitely changed the chemical environment of the oviposition site for which the chemoreceptors of the ovipositor might not have stimulated for oviposition. Nanda and his co-workers (1999) observed higher rate of oviposition by BPH female on susceptible TN 1 than on resistant accession. Shu *et al.* (2009) investigated that

application of zinc down regulated vitellogenin gene as a result *Spodoptera litura* females laid fewer eggs in laboratory conditions. In our study we also observed that WBPH females laid fewer eggs on TN 1 treated with zinc fertilizers which in turn inferred induced antixenosis effect of zinc. Besides fewer egg laying, hatchability was also reduced in zinc treated plants as compared to untreated TN 1 plants. Poor hatching of WBPH eggs in rice plant exposed to zinc has also been studied earlier by Rath (1995). Therefore, the present observation lies in conformity with opinion of above scientists.

#### **5.4 Induced effect of zinc for antibiosis against WBPH**

Various antibiosis parameters were studied against WBPH to ascertain the possible induced effect of zinc. The pot culture studies on nymphal survival, nymphal development, sex ratio, growth index, adult longevity, population build up, feeding potential and weight gain of WBPH etc. were conducted and the data of above experiment has been presented in Table 18.

Nymphal survival was tremendously reduced in T<sub>6</sub>, T<sub>7</sub>, T<sub>5</sub> and T<sub>8</sub> treatments (Table 18) which accounted for 56 per cent in T<sub>8</sub> to 44 per cent in T<sub>6</sub>. All other zinc treatments also reduced the nymphal survival when compared against the control. Reduction in nymphal survival was due to poor nutritional quality of the zinc treated plants than untreated plants. Nymphal period enhancement is one antibiosis parameter. In the present study, WBPH nymphs lived for 13.90 days in control, whereas, in all the zinc treatments, the nymphal duration was enhanced by 0.1 to 3.40 days. Increase in nymphal period in zinc treated plants might be attributable to poor ingestion and assimilation resulting in poor accumulation of degree days and to achieve the required degree days to be converted to next stage, the nymphs of zinc treated rice plant may have taken more days. Enhancement of nymphal duration on zinc treated rice plants also has been studied earlier by Pati (2002) and Rath (2004).

Since the growth index is the ratio of nymphal survival and nymphal duration, the same was observed to be least in T<sub>6</sub> and highest in T<sub>9</sub> (Table 18). Resistant varieties being unfavourable for insects due to low nutritional quality, favours a lower growth index. Similarly, in the present study it was evident that zinc treated rice plants favoured lower growth index of WBPH and this might be attributable to either improper nutrition or lack of vital nutrients or formation of toxic substances. Lower

growth index of WBPH fed on zinc treated rice plants has been earlier demonstrated by Pati (2002) and Rath (2006). It was also observed from the study that the zinc treated plants favoured more male forms than females when compared with control. Among the zinc treatments, the treatment T<sub>6</sub> and T<sub>7</sub> resulted in extremely more male forms (0.88 and 0.93 F: M). Gunathilagaraj and Chelliah (1991) have also reported production of more male forms in resistant rice accessions due to cumulative effect of lower fecundity as well as nymphal survival through several pest generations. Therefore, the present finding lies in conformity with the finding of above authors.

Adult longevity (both male and female) was observed to be less in all the zinc treatments; spectacular being in T<sub>6</sub> and T<sub>7</sub> when compared with the control treatment. Thus, it was clear that control rice plants were nutritionally suitable for which the life span of WBPH adults was completed normally while, in zinc treated plants, there was reduced adult duration. Hence, it is perhaps the zinc supplementation which definitely might have altered the nutritional status of rice plants treated with zinc fertilizers, for which the adult life span of both male and female WBPH was less in zinc treatments. Lower adult longevity of WBPH on resistant varieties has been visualized by Gunathilagaraj and Chelliah (1991) and particularly shortening of adult life span in WBPH of rice under zinc umbrella has been reported by Rath (1995) and therefore, the present finding is well supported by the above findings.

Preference/Non-preference of a variety by the insect is greatly strengthened by the population load of the insect on the variety within a stipulated time along with the reaction of the insect on a susceptible host. While susceptibility (more insect population) is the degree of suitability of the plant variety by insect, there the degree of resistance of other comparable varieties is the degree of unsuitability by same insect. In the present study, population build up of WBPH was found to be highest in the control treatment (79.2 per hill) and the population was less in all other treatments. Even the treatment T<sub>6</sub> supported nearly 60 per cent less population when compared with control. Earlier workers *viz.*, Rath and Misra (1998) and Rath (2004) also have witnessed low population of WBPH in rice being subjected to zinc application.

The feeding potential study also envisaged more feeding by WBPH females on control plants as the amount of honey dew secreted was highest with regards to control plants. More feeding is the indication of balanced nutritional status of the plant and therefore, less feeding by WBPH on treated rice plants indicated a definite nutritional

imbalance. Thus, it was clear that nutritional imbalance was effected due to different graded doses of zinc and subsequent differential uptake of zinc by rice plants. Gunathilagaraj and Chelliah (1985) have also observed reduced feeding potential of WBPH females on resistant rice variety than on susceptible ones. Therefore, the present finding corroborate strongly with the findings of the above authors.

Reduced weight gain in feeding insect is also considered as an important antibiosis factor of the host plant. Weight is directly correlated to feeding and assimilation. In the present study, we noticed that weight gained by both WBPH male and female adults was highest in control treatment, whereas, the adults feeding on zinc treated rice plants suffered. Though, there was gain in weight of all the individuals exposed to different treatments, yet, the rate of weight gain was poor in all the treatments as compared to control treatment. Again, it was also visualised that the rate of weight gain was more in female adults than in males. Similar studies have been reported by Sogawa (1973) and Ramulamma (2014).

### **5.5 Induced effect of zinc for tolerance against WBPH**

Pot culture studies were also conducted to assess the induced tolerance effect in rice plants in terms of duration to wilting, functional plant loss index (FPLI) and loss in plant biomass being influenced by zinc application (Table 19). Enhancement in duration of wilting of rice plants under exposure of higher pest load above ETL is considered as a tolerance mechanism (Jhansilakshmi *et al.*, 2012). Alagar and Suresh (2007) have also observed that resistant variety took 27-31 days for wilting as compared to susceptible TN1 (18.2 days). In the present study, we observed that when TN 1 plants died completely in 17.33 days, at same population load wilting started in T<sub>6</sub>, T<sub>5</sub> and T<sub>7</sub> treatments nearly 10 days later. This indicated a probable withstanding capacity of zinc treated plants against high hopper infestation. Hence, the present finding is in line of conformity with the above authors.

It was also observed that the FPLI was minimum (27.80 %) in T<sub>6</sub> which was significantly not different from rest of the treatments except the basal treatments. Highest loss index was seen in control (69.51 %). Plant biomass loss is directly proportional to WBPH feeding activity and less loss in T<sub>3</sub> to T<sub>8</sub> even at high pest load may be attributable to less insect activity and subsequent less feeding or a compensatory mechanism induction due to zinc application. Alagar and Suresh (2006) also have witnessed a similar phenomenon.

## 5.6 Impact of zinc on biochemical parameters of rice plants

Various biochemical parameters sampled from 60 DAT rice plant from pot culture experiment were analysed through standard procedure and the result have been presented in Table 20 (a and b). It was visualized from the table that total chlorophyll content in T<sub>7</sub> was increased by 77.88 per cent over control, whereas, the treatment T<sub>6</sub> accounted for 70.19 per cent increase. Zinc is one important component involved in synthesis of chlorophyll in rice (Aravind and Prasad, 2004). Ayad *et al.* (2010) also described that application of zinc triggered activity of enzyme related to biosynthetic pathway for chlorophyll synthesis. Therefore, the present finding corroborate with the finding of above authors.

It was also visualized that total phenol content increased in all the zinc treatments when compared against control. The treatment T<sub>6</sub>, T<sub>7</sub> and T<sub>8</sub> were responsible for 181.82 and 163.64 per cent increase in phenol content over control treatment. Zinc enhanced the activity of phenylalanine ammonia lyase (PAL) (Wadhwa *et al.*, 2014) because it acts as a cofactor for synthesis of PAL. PAL catalyses the biosynthesis of phenolics (Punithavalli *et al.*, 2013) and Southerton (1990) also observed direct correlation of induction of PAL activity with phenol content. In the present finding phenol amount was enhanced in treatments comprising of both basal and foliar spray. Increase in phenol content was attributable to increase in PAL activity. Hence, our finding is in conformity with the finding of above authors.

The free amino acid content was observed to be low in treatment T<sub>6</sub> (9.74 % less than control). Though, the decrease in free amino acid content was statistically less in all the zinc treatments, the free amino acid content was at par with control. Thus, it can be inferred that the presence of free amino acid perhaps did not contribute as an antibiosis factor strongly. However, Vanitha *et al.* (2011) have reported that in susceptible TN 1, free amino acid content was higher than resistant rice accession i.e. Ptb 33, Ast-16 and Basmati-370.

The total soluble protein was observed to be highest in T<sub>6</sub> followed by T<sub>8</sub> accounting for 19.98 and 19.06 per cent increase over the control treatment. In rest of the treatments with zinc, there was 1.48 to 13.36 per cent increase in total soluble protein content. Punithavalli *et al.* (2013) witnessed that there was a slight decrease in the protein content invariably in the leaf folder infested plants as compared to healthy plants. Thus, our finding is in line of conformity with the above author.

The total soluble sugar was reduced in almost all the zinc treatments as compared to control. It seems that only T<sub>6</sub>, T<sub>7</sub>, T<sub>8</sub> and T<sub>5</sub> treatments, which were at par produced less total soluble sugar than other zinc treatments, which were more or less similar to control. Combination of zinc treatments both as basal and foliar spray was superior to either sole basal application or foliar spray. Depletion of total soluble sugar was resultant effect of WBPH feeding and the correlation study also indicated that the WBPH population and total soluble sugar were positively correlated. Rath *et al.* (1999), Rath and Mishra (1998) and Pati (2002) have also witnessed a similar effect in rice against WBPH.

The proline level generally get enhanced with enhancement in biotic stress (Fabro *et al.*, 2004) but in the present finding zinc application reduced the WBPH number, for which there was decrease in proline level in all the treatments as compared to control. It was witnessed that the treatment T<sub>6</sub> accounted for 14.63 per cent less proline than control. Similar phenomenon has been studied by Derakhshani *et al.* (2011).

### **5.7 Effect of zinc application on enzymatic activity in rice infested by WBPH**

The activity of super oxide dismutase (SOD) was analysed and the data revealed that SOD activity was enhanced in all the zinc treatments as compared to control. Within zinc treatments, highest activity was observed in T<sub>6</sub> (4.21 Units/min/mg protein) (Table 21), which was significantly different from rest of the treatments. Zinc treatment comprising either sole basal/foliar spray recorded comparatively lower SOD activity than combination of basal/foliar treatments. Increase in zinc content also increased SOD activity and this phenomenon has also been observed by Mathpal *et al.* (2015). Similarly, in the present finding peroxidase (POD) activity was also observed to be high in all the zinc treatments as compared to control and within the zinc treatments, T<sub>6</sub> supported highest POD activity remaining at par with T<sub>5</sub> and T<sub>7</sub> treatments. POD is also one defensive enzyme whose accumulation in infested rice was observed after 1 day of BPH infestation (Alagar *et al.*, 2007). Higher is the concentration of antioxidative enzymes in the plant system; lower is the biotic stress (Punithavalli *et al.*, 2013). Our finding derived ample support from the finding of the above authors. The correlation coefficient between population build up and POD activity was also observed to be negatively correlated with high level of significance

(Table 25). Such correlation study also strengthened our above finding. The PAL activity was also high in all the zinc treatments as compared to control. This has also been confirmed through correlation study ( $r = -0.91$ ). The increase in PAL activity in rice that reduced leaf folder infestation was also observed by Punithavalli *et al.* (2013). However, the flowchart of role of zinc in inducing defense mechanism in rice against WBPH is presented in Figure 16.

## **5.8 Zinc content of rice plant at maximum insect activity and its correlation with various parameters**

### **5.8.1 Zinc content of rice plant**

The data on zinc content of rice plant at maximum insect activity has been presented in Table 22. It was quite conspicuous that zinc content was more in all the zinc treatments as compared to control. The treatments like T<sub>6</sub> and T<sub>7</sub> recorded more zinc content within all zinc treatments accounting for 26.75 and 22.43 per cent increase in zinc content. The zinc content of the pot culture plant samples also exhibited a similar response as that of field data, where, T<sub>6</sub> and T<sub>8</sub> caused each of 33.12 per cent increase in zinc content as compared to control. Higher zinc content due to application of Zn EDTA and ZnSO<sub>4</sub> in rice has been evidenced by Karak *et al.* (2005). Enhancement of Potassium content in plants confers resistance to many phytophagous insects. In the present study, the increase in potassium content due to zinc application might have acted synergistically as a result of which application of zinc to rice plants induced resistance phenomenon against WBPH. Srivastava *et al.* (2016) also observed that foliar application of zinc increased the zinc and potash concentration in flag leaves and straw of rice significantly because zinc fertilizer enhanced the utilization of potassium content of soil and ultimately resulting in higher uptake of potash. Thus, our finding corroborate with the findings of above authors.

### **5.8.2 Correlation between zinc content vs. plant growth parameters and WBPH population (field sample)**

The correlation between zinc content and various plants growth characters studied over three seasons indicated that all the plant growth parameters like number of tillers, plant height, number of panicle/m<sup>2</sup>, number of grains/panicle and grain yield were all positively and significantly correlated to zinc content of the plant sample taken

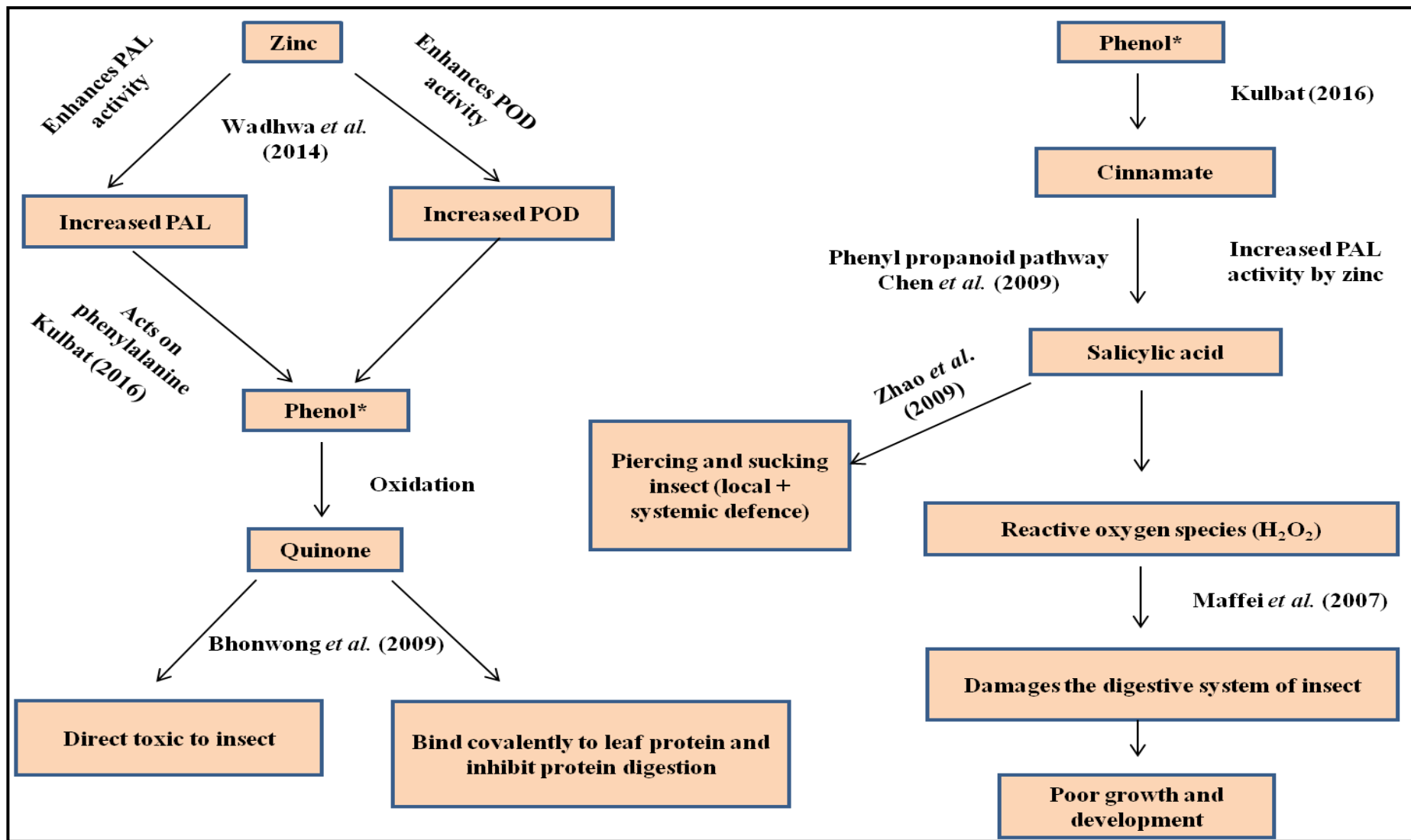
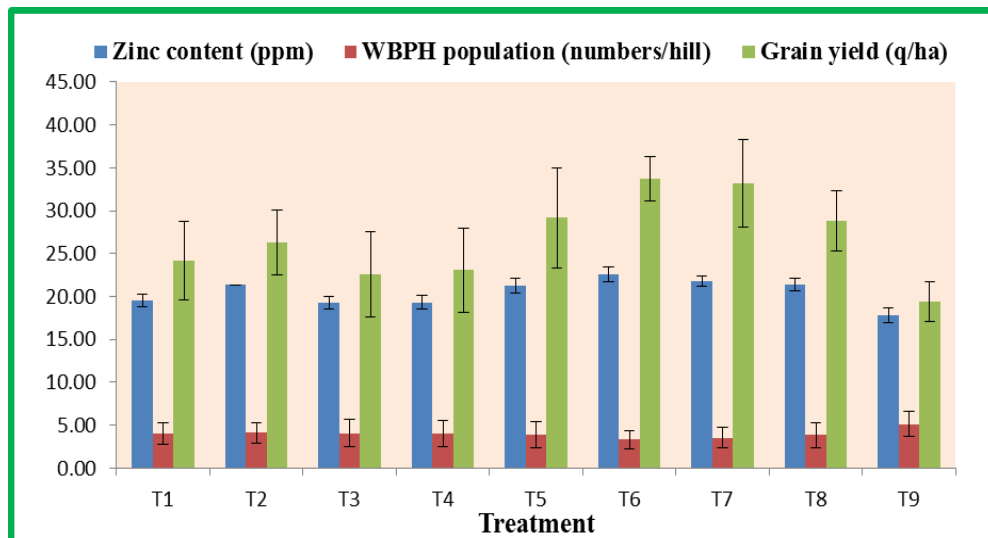


Fig. 16: Flowchart for role of zinc in induced defense in rice to WBPH

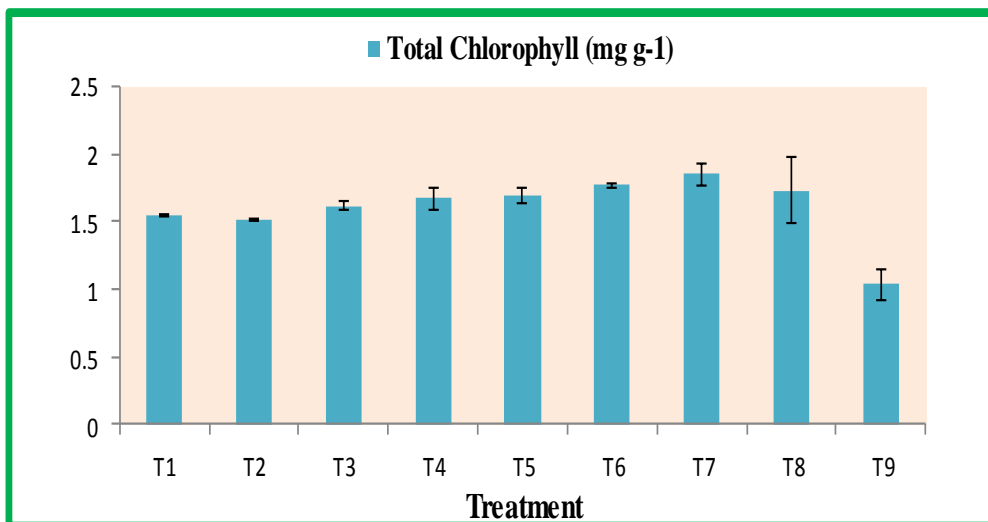
from field study at maximum insect activity (60 DAT) (Figure 17) except for zinc content vs. number of panicle/m<sup>2</sup> during *kharif* (though positively related not significant). Several workers *viz.*, Ghasal *et al.* (2015), Silviya and Stalin (2017) and Firdous *et al.* (2018) have also studied that increased zinc content also increased various plant growth parameters. The correlation between zinc content and WBPH population revealed a strong negative relationship which indicated that increase in zinc content decreased WBPH population substantially. Reduction in WBPH number due to application of zinc has also been observed by Rath (2004) and Dash and Mishra (2009).

### **5.8.3 Correlation between zinc content vs. plant biochemical parameters (pot culture)**

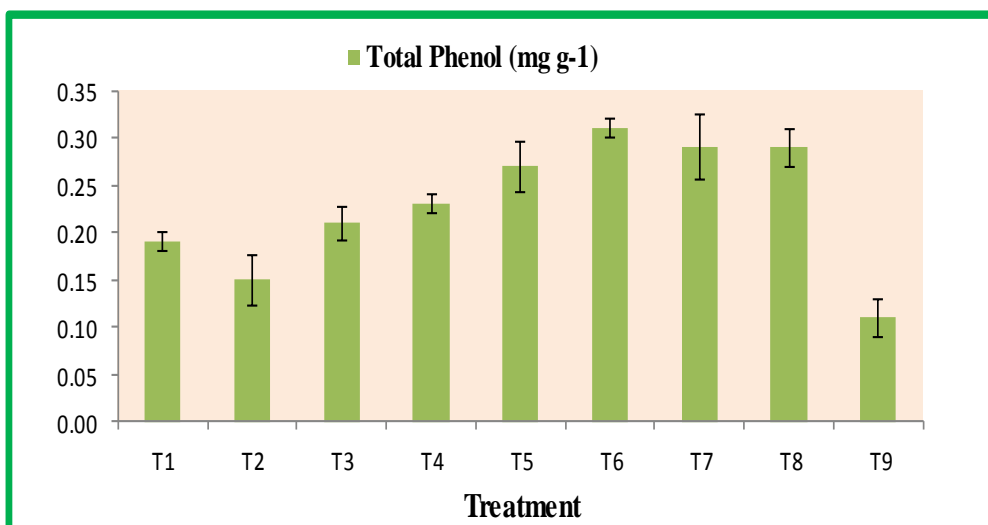
Increase in zinc content in rice plant increased the total chlorophyll content ( $r = 0.73$ ) (Figure 18). Ayad *et al.* (2010) opined that zinc plays a vital role in triggering certain enzymes responsible for chlorophyll biosynthetic pathway. Hence, our finding corroborate with the finding of above author. Similarly, zinc content was observed to be highly and positively correlated to total phenol (Figure 19) and total soluble protein content. Zinc enhances PAL activity which in turn favours more phenol content. This has been studied by Prasad *et al.* (2010) and Punithavalli *et al.* (2013) and our investigation is in line of conformity with above authors. Similarly, increased zinc favouring increased total soluble protein content has also been studied by Roohani *et al.* (2013). A strong negative correlation was evidenced between zinc content and free amino acid (Figure 20) and total soluble sugar (Figure 21). Similar finding has also been observed by Kitagishi and Obata (1986) and Derakhshani *et al.* (2011). With regards to correlation between zinc and proline content, a negative correlation was observed which was quite significant. This indicated that improved zinc content decreased the biotic stress (WBPH population) and decreased insect activity was responsible for decreased proline content. Derakhshani *et al.* (2011) also studied a similar phenomenon of negative relation between zinc and proline content. With respect to zinc and various enzymatic activities, it was visualized that increase in zinc content was positively related to SOD, POD and PAL activity (Figure 22). A number of scientist *viz.* (Mathpal *et al.*, 2015- SOD), (Wadhwa *et al.*, 2014- POD and PAL) have also studied a similar phenomenon that supported our finding.



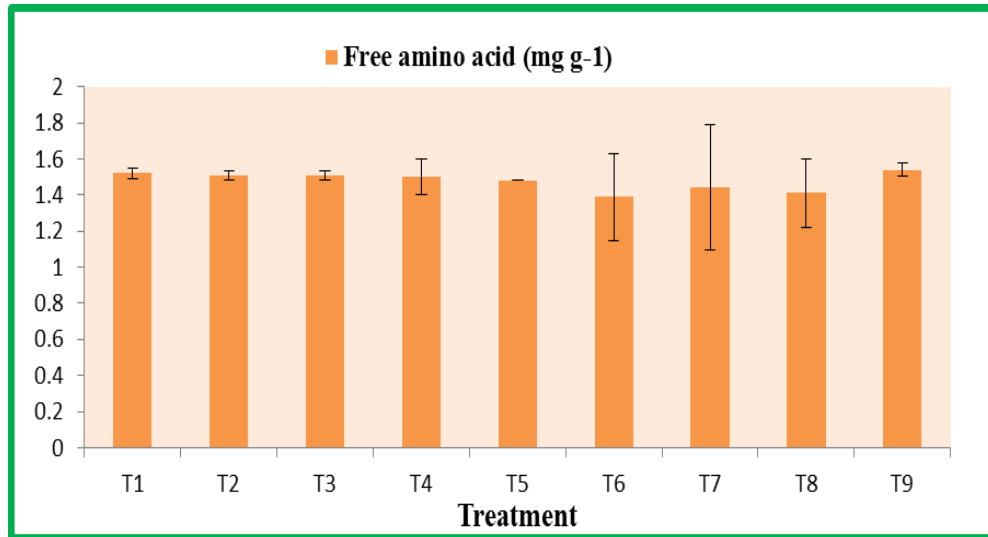
**Fig. 17: Influence of zinc application on rice plant in relation to WBPH population and grain yield under field condition**



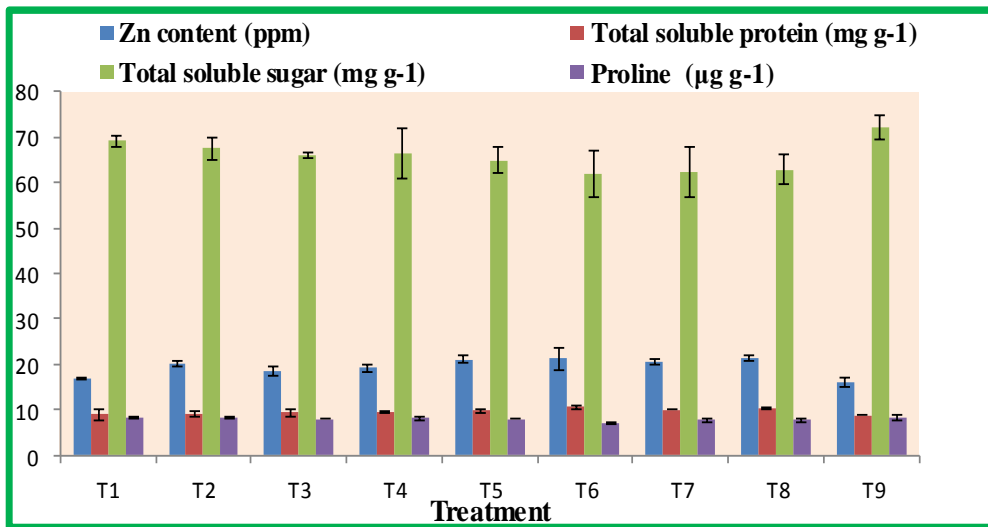
**Fig. 18: Effect of zinc application on total chlorophyll content of rice plant**



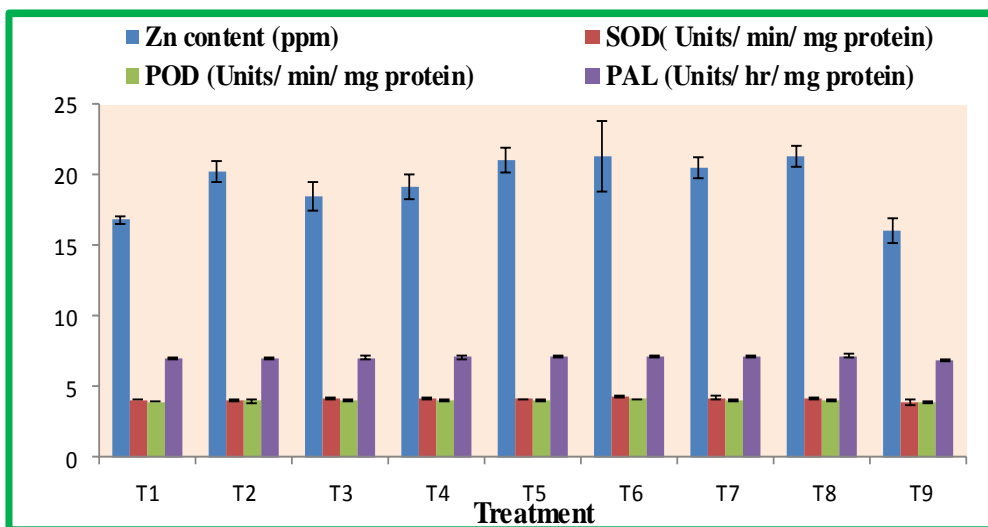
**Fig. 19: Impact of zinc application on total phenol content of rice plant**



**Fig. 20: Role of zinc application on free amino acid content of rice plant**



**Fig. 21: Impact of zinc application on various biochemical parameters of rice plant**



**Fig. 22: Role of zinc application on various enzymatic activity of rice plant**

#### **5.8.4 Correlation between WBPH population vs. various plant biochemical parameters**

The WBPH population at its highest activity was correlated with biochemical parameters of sample taken from rice plant at that period. It was evident that the WBPH population was negatively correlated to total chlorophyll ( $r = -0.96$ ), total phenol ( $r = -0.90$ ), total soluble protein ( $r = -0.83$ ), SOD ( $r = -0.88$ ), POD ( $r = -0.90$ ) and PAL ( $r = -0.91$ ), respectively.

Watanabe and Kitagawa (2000) studied that both nymphs and adults of WBPH feed on the phloem of rice plant and causes loss in chlorophyll contents and photosynthetic rate. Therefore, control treatment devoid of zinc application harboured maximum WBPH population (Table 18) and minimum chlorophyll content (Table 20a) than rest of the treatments. Thus, our finding corroborate with the findings of above author.

Phenol content acts as chemical barrier against insect pest damage (Ashrith *et al.*, 2017). Mohan *et al.* (1988), Rath and Misra (1998), Pati (2002), Chandramani *et al.* (2009), Deepa *et al.* (2016) and Ashrith *et al.* (2017) have reported that high phenol content in rice is negatively correlated with the incidence of the WBPH and BPH. Therefore, the present finding is in close conformity with the findings of the above authors.

Punithavalli *et al.* (2013) reported that there was a slight decrease in the protein content invariably in the leaf folder infested plants as compared to healthy plants. Ashrith *et al.* (2017) witnessed a negative correlation between total soluble protein and plant hoppers population and considered it as a possible resistance factor in rice against hoppers. Thus, our finding is in line of conformity with the opinion of the above authors.

Infestation induced reactive oxygen species (ROS) that imparts resistance against the insect is ideal but when ROS concentration increases in the plant system, the SOD activity is triggered which ultimately scavenge the ROS and rescue the plants from autocidal effect. The SOD activity was maximum in T<sub>6</sub> (Table 21), where the population build up was low. Thus, the present finding lies in conformity with the

statement that higher was the SOD activity, better was the oxidative stress tolerance (Slooten *et al.*, 1995).

Punithavalli *et al.* (2013) observed lower activities of antioxidative enzymes such as POD and PAL in susceptible genotypes of rice leaf folder. As the control treatment harboured highest population and had lowest POD and PAL activity (Table 21) than all other zinc treated plants and, therefore, a negative correlation existed between population build up with POD and PAL.

Besides the above parameters, population build up was positively correlated with free amino acid ( $r = 0.82$ ), total soluble sugar ( $r = 0.94$ ) and proline ( $r = 0.82$ ). Muhammad Salim (2002) and Chen (2009) reported that sugar content act as feeding stimulants for sap feeders and report do exist that the chemical composition of the rice plant can influence BPH feeding behaviour. Pati (2002) reported that WBPH population was significantly and positively correlated with total sugar and amino acid content. Therefore, WBPH resistance factor was attributed to low sugar and amino acid content. Thus, our finding supports the findings of above authors.

Accumulation of proline under stress condition is a common phenomenon which can go as high as 100 times as that of non-stressed condition (Verbruggen and Hermans, 2008). Therefore, as the WBPH population build up was high in control treatment (Table 18), the accumulation of proline was also more. But with supplementation of zinc, the plant might have developed induced mechanisms of resistance resulting in less stress for which proline content in zinc treatments was low. Therefore, the present investigation is supported by the opinion of the above authors.

## **5.9 Protein profiling**

Plants respond to insect attack through a variety of defense mechanisms which may be either morphological adaptation (trichomes, pubescence, waxy cuticle), or elicitation of biochemical and molecular mode of defense systems. Host plant resistance can be developed by appropriate breeding strategies. Besides, adequate level of resistance can be engineered genetically to build up endogenous defense biomolecules within the host plants to confer resistance to insects. Many often, in absence of above inherent insect resistance mechanisms, induced resistance can be

elicited in the host plants by use of chemical elicitors of secondary metabolites, potassic fertilizers or even chemical compounds containing silicon, zinc and ferrous.

Zinc is needed for plant growth and resistance to biotic and abiotic stresses. Sufficient accumulation of zinc (either through uptake from soil or by foliar spray) followed by its cellular sequestration make the plants climatically more smart (abiotic stress tolerant). Zinc finger proteins are among the most abundant proteins in eukaryotic genomes. Their functions are extraordinarily diverse. It includes DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly, and lipid binding. Insect infestation is reported to be related to the up-regulation of two zinc finger transcription factors in potato (Lawrence *et al.*, 2014).

In the present context, protein profile of various treatments exposed to different zinc fertilizer in pot culture experiment were analysed by Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) method to ascertain whether zinc is responsible for production of any new defensive protein against WBPH or not. Zinc serves as a co-factor for more than 300 enzymes in plant and animal system (Marreiro *et al.*, 2017). Altogether, nineteen polypeptide bands were revealed in response to extraneous application of zinc in form of basal and foliar application of ZnSO<sub>4</sub> and Zn EDTA. Polypeptide bands i.e. B1 (97.4 kDa), B2 (90 kDa), B4 (78 kDa), B8 (40.2 kDa), and B13 (27.3 kDa) in the zymogram are monomorphic over all the treatments and control indicating their expression independent of the zinc application. It was observed that polypeptide bands of 29.0 kDa, 35.0 kDa, 56.8 kDa and 85.5 kDa were absent in basal application of ZnSO<sub>4</sub>, but induced in all treatments and even in control. In contrast, 33.0 kDa and 25.1 kDa polypeptides were induced by basal application of ZnSO<sub>4</sub>, but down-regulated in all treatments including control. Zinc uptake in case of basal application is determined by presence of Zn-transporter genes in root cells followed by transport to stem and foliage, while foliar application can bypass such genetic system and avail zinc directly to the enzymes required for plant growth and metabolism (Zhao *et al.*, 2014). Basal application of Zn EDTA and also foliar application of ZnSO<sub>4</sub> alone produced a polypeptide band of 20.1 kDa. Two polypeptide bands *viz.*, 37.0 kDa and 66 kDa were induced in ZnSO<sub>4</sub> basal, but not expressed in control as well as in response to its foliar spray alone or its additional application as foliar spray. Even these two polypeptide bands were also not expressed in Zn EDTA

basal or foliar spray alone, but induced by combination of basal + foliar spray of Zn EDTA as well as combination of basal ZnSO<sub>4</sub> + foliar Zn EDTA or vice versa. The low molecular weight proteins ranging from 14.3-25.1 kDa were clearly absent in the control, but zinc application in form of above sources as basal or foliar treatment elicited biosynthesis of new polypeptide bands. For instance, a polypeptide band (14.3 kDa) was noticed in all the zinc treatments except ZnSO<sub>4</sub> basal. Besides, foliar spray of Zn EDTA in combination with basal application of either ZnSO<sub>4</sub> or Zn EDTA induced new polypeptide band at 23.6 kDa. In the present investigation, elucidation of the induced polypeptide bands revealed in response to various treatments of zinc-application may throw light for better understanding about the biochemical basis of induced host plant resistance.

WBPH is a dreadful sucking insect of rice and it usually attack at culm base of rice plant at tillering and flowering stage causing drastic reduction in crop growth and seed yield (CRIDA, 2019). Zinc application either as basal and/or foliar application reduced the population build up of WBPH ranging from 31.40 to 53.60 insects/hill as against control (79.20 insects/hill). Combination treatment of basal and foliar application of zinc formulation in the form of Zn EDTA (T<sub>6</sub> : T<sub>2</sub> + T<sub>4</sub>) resulted maximum dividend followed by T<sub>7</sub> (T<sub>1</sub> + T<sub>4</sub>) and T<sub>8</sub> (T<sub>2</sub> + T<sub>3</sub>) in term of decrease in WBPH population. It is worth to note that the most responsive zinc combination treatment (T<sub>6</sub>) elicited highest number (15) of polypeptide bands against ten normal protein bands in the control. Expression of five new polypeptide bands at 66.0, 37.0, 23.6, 15.8 and 14.3 kDa induced by T<sub>6</sub> can be considered as biochemical basis of induced resistance in rice against WBPH. Among these, 66.0, 37.0, 23.6 and 14.3 kDa protein bands were induced by T<sub>7</sub> and 66.0, 37.0, 15.8 and 14.3 kDa protein bands induced by T<sub>8</sub>, were common to that of T<sub>6</sub>, which were new types and not expressed in control treatment. Further, the soluble protein profiling study revealed that 66.0 kDa, 37.0 kDa and 14.3 kDa polypeptide bands were significantly induced and commonly shared in T<sub>6</sub>, T<sub>7</sub> and T<sub>8</sub> that recorded lower WBPH population. However, 23.6 kDa polypeptide band induced only in T<sub>6</sub> and T<sub>7</sub> seems to have greater role in manifestation of induced resistance to WBPH in rice. Induction of a defense related protein (53 kDa), due to leaf folder infestation in resistant and moderately resistant rice varieties have been reported by Das *et al.* (1999). Similarly, Sinha *et al.*, (2005) and Punithavalli *et al.* (2013) have also noticed enhanced expression of a high molecular weight (> 97 kDa)

protein in all the genotypes but there was an increased induction of a 38 kDa protein in leaf folder infested resistant rice genotypes, which was absent in uninfested plants. They reported these as a defense related proteins. Therefore, the present finding lies in conformity with the finding of above authors.

Further, clustering pattern/ dendrogram based on pair-wise similarity coefficient values revealed that T<sub>1</sub>, T<sub>9</sub> (Control), T<sub>6</sub>, T<sub>7</sub> and T<sub>8</sub> formed single treatment cluster while, both T<sub>2</sub> and T<sub>3</sub> together and also T<sub>4</sub> and T<sub>5</sub> combinedly formed separate clusters at 100 per cent phenon level. Had there been fine tuning of the protein profiling T<sub>2</sub> and T<sub>3</sub> might be separated and this difference could have been interpreted in terms of per cent damage difference between T<sub>2</sub> and T<sub>3</sub>. Similarly, T<sub>4</sub> and T<sub>5</sub> could have been differentiated as we have got difference between these in field and pot culture experiment. However, the difference between T<sub>4</sub> and T<sub>5</sub> was not so much vigilant as compared to their effect with either of T<sub>6</sub>, T<sub>7</sub> or T<sub>8</sub>, which revealed higher induction of host plant resistance to WBPH.

## SUMMARY AND CONCLUSION

The induced resistance effect of zinc applied in the form of ZnSO<sub>4</sub> and Zn EDTA as basal or foliar spray or in combination on rice against white backed plant hopper (WBPH) both under field trials (*kharif*, 2016, summer, 2016-17 and *kharif*, 2017) and pot culture experiments (*kharif*, 2017) were conducted at the Central Research Farm, Department of Entomology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar. The details of various experiments conducted, results obtained and validation of the result through discussion has already been presented in respective chapters. The summary of the entire project and the conclusion derived is presented in this chapter.

Application of zinc to the rice crop was instrumental in curbing the WBPH population as compared to control in all the test seasons. Among various treatments, T<sub>6</sub> (Zn EDTA basal @ 40 kg/ha + Zn EDTA foliar spray @ 0.8 % twice at 30 and 45 DAT) was the best treatment that could reduce WBPH number by 66.15 per cent which was outstanding. Closely followed by T<sub>6</sub>, the treatment T<sub>7</sub> (ZnSO<sub>4</sub> basal @ 25kg/ha + Zn EDTA foliar spray @ 0.8% twice at 30 and 45 DAT) caused 60.84 per cent elimination of the target pest.

Application of zinc not only curtailed WBPH population on rice, also enhanced various plant growth and yield attributing parameters. Zinc supplementation increased the tiller numbers per hill (around double than control), plant height, number of panicle/m<sup>2</sup> and number of grains/ panicle. Regardless of the mode of application, basal application of ZnSO<sub>4</sub> was at par with combination treatments. Thus, the phytotonic effect in rice owing to zinc application was quite obvious. The grain yield was also observed to be more in all the zinc treatments (16.54 to 74.17 % increase) than the control treatment and within the zinc treatments, T<sub>6</sub> performed better than others. One of the major contributions of the zinc was improved photosynthesis through increased chlorophyll production.

Studies conducted on antixenosis mechanism induced by zinc on rice revealed that zinc treated rice plants attracted less number of nymphs and adults of WBPH as compared to control. Similarly, the female adults of WBPH laid more number of eggs in control plants as compared to zinc treated plants. We opined that the orientation of nymphs and adults of WBPH to varied degree in zinc treated and untreated plants were

due to both olfactory and gustatory response. Less oviposition (nearly 60 %) in T<sub>6</sub> having both basal and foliar treatments of zinc was a clear cut demonstration of induced antibiosis effect of zinc because the untreated control plants favoured more oviposition. Not only the rate of oviposition but also the hatchability was less in zinc treated plants as compared to control. A number of earlier workers have also witnessed less oviposition by WBPH on resistant rice accessions. Reduced oviposition was attributable to unfavourable chemical environment at the tissue level probably caused by zinc application.

Various antibiosis parameters strengthening induced effect of zinc on rice was also visualized conspicuously. Nymphal survival of WBPH was drastically reduced in all the combined applications of zinc than sole application of zinc either as basal or foliar spray. Irrespective of zinc dose and application methods, all the zinc treatments reduced nymphal survival as compared to control and this phenomenon was attributable to poor nutritional quality of the zinc treated plants. Increase in phenol content of the rice plants due to zinc application coupled with high phenylalanine ammonia lyase (PAL), peroxidase (POD) and superoxide dismutase activity (SOD) and reduced proline content were responsible for low nymphal survival. Enhancement of nymphal period on zinc treated rice plants was also pronounced when compared against control. Host plant unsuitability due to poor nutrition status might be the cause of elongation of nymphal duration.

Since, growth index of WBPH was the ratio of nymphal survival and nymphal duration, the control plants favoured higher growth index of WBPH nymphs than all the zinc treated rice plants. Visualization of more male forms in zinc treated plants particularly in T<sub>6</sub> was another pronounced phenomenon of induced antibiosis effect of zinc. Besides, we also observed reduced life span of both male and female adults of WBPH on zinc treated rice plants. All those antibiosis parameters were definitely caused due to poor nutritional status of zinc treated plants.

Population build up study confirmed the plant nutritional status. The untreated TN 1 rice plants supported high WBPH population as compared to the rice plants treated with zinc. This indicated that untreated TN 1 plants were more suitable than the treated plants as the population in untreated plant was nearly double than that of treated plants. This study was further strengthened by the honey dew excretion test as a result of feeding potential. WBPH females excreted less honey dew fed on treated rice plants

as compared to untreated plants. Reduced excretion was attributable to reduced feeding and reduced feeding was observed because of poor nutritional status due to zinc application. Effect of feeding was also assessed in terms of body weight gain both in female and male adults of WBPH. There was significant body weight gain in WBPH adults that were fed on untreated rice plants than the treated ones. Hence, all the antibiosis parameters were quite pronounced in zinc treated rice plants than the untreated ones and therefore, it can be inferred that zinc supplementation induced antibiosis mechanism in rice plants against WBPH.

The results of the induced tolerance study were also quite conspicuous. While, the same pest load was maintained in all the treatments, zinc treated plants particularly T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub> took longer period to exhibit wilting symptoms, whereas, the untreated TN 1 plants completely withered much before. The functional plant loss index (FPLI) was also least in T<sub>6</sub> and other zinc treatments as compared to control. Lower FPLI is a measure of tolerance mechanism and the present investigation with induced effect of zinc well supported the above statement.

We have also analysed various biochemical constituents of rice plants taken from pot culture studies. Plant samples were analysed for total chlorophyll, total phenol, total free amino acid, total soluble protein, total soluble sugar and proline content. It was observed that while application of zinc increased total chlorophyll content, total phenol content, total soluble protein content, the same zinc reduced the total free amino acid, total soluble sugar and proline content in zinc treated plants. Both increase and decrease in respective biochemical parameters were the indicators of induced antibiosis mechanism in rice plants against WBPH.

Besides the analysis of biochemical constituent, the enzymatic activity of rice plants exposed to zinc treatment and without zinc treatment at maximum WBPH infestation was also determined. It was clearly evident that both SOD and POD activity were enhanced in all the zinc treatments compared to control. Both basal and foliar spray treatments recorded higher SOD activity than sole treatment. Both these enzymes are defensive enzymes in the plant against biotic stress. In addition to these above two antioxidative enzymes, PAL activity was also noticed to be high in zinc treated plants than the untreated plants.

To determine the correlation between zinc content and various plant morphological and biochemical parameters under study, zinc content of the plant sample was also estimated. Zinc content was substantially enhanced in all the treatments when compared against the control. Zinc content was found to be positively correlated to all the plant growth parameters *viz.*, plant height, number of tillers/hill, number of panicles/m<sup>2</sup>, number of grains/panicle at high significance level which strengthened the phytotonic activity of zinc in rice plants. Further, zinc content was significantly and positively correlated to total phenol, total chlorophyll, total soluble protein while, the same was significantly and negatively correlated to total free amino acid, total soluble sugar and proline content. Zinc content was also witnessed to be positively correlated to SOD, POD and PAL activity.

The WBPH population recorded from the pot culture experiment that coincided with the crop stage of field condition with highest pest activity was also correlated to various plant biochemical parameters. While, WBPH population was negatively correlated to total chlorophyll content, total phenol content, total soluble protein content, SOD activity, POD activity and PAL activity, at this time it was positively correlated to total free amino acid content, total soluble sugar content and proline content.

The field and pot culture experiment was also supported by protein profiling study. Various combined application of ZnSO<sub>4</sub> and Zn EDTA produced specific polypeptide bands of 66.0 kDa, 37.0 kDa and 14.3 kDa, which was not seen in control. Since, the polypeptide band 23.6 kDa was synthesized in T<sub>6</sub> (Zn EDTA basal @ 40 kg/ha + Zn EDTA foliar spray @ 0.8 % twice at 30 and 45 DAT) and T<sub>7</sub> (ZnSO<sub>4</sub> basal @ 25kg/ha + Zn EDTA foliar spray @ 0.8% twice at 30 and 45 DAT) which supported least WBPH population, the above protein i.e. 23.6 kDa was adjudged as most defensive protein responsible for induced resistance in rice plant to WBPH.

From the entire investigation, it can be concluded that basal application of Zn EDTA @ 40 kg/ha along with its foliar spray @ 0.8% twice at 30 and 45 days after transplanting (T<sub>6</sub>) was the best treatment in inducing resistance in rice against WBPH through sustained supply of beneficial micronutrient (zinc), which also enhanced the desirable yield attributing characters of rice plants. Similarly, in consideration to antixenosis parameter, this element reduced the nymphal alightment, adult alightment,

total number of eggs laid by the female and per cent hatching of eggs. With respect to antibiosis parameters, T<sub>6</sub> reduced the nymphal survival, sex ratio, growth index, adult longevity, population build up and weight gain of adults along with feeding potential, whereas, nymphal period and male formation was enhanced in the same treatment as compared to control. As regards to tolerance mechanism, zinc application (T<sub>6</sub>) reduced the functional plant loss index and enhanced the wilting period. Various biochemical constituents like total chlorophyll, total phenol and total soluble protein content were increased due to zinc application, whereas, total free amino acid, total soluble sugar and proline content were reduced. The enzymatic activities of SOD, POD and PAL were triggered in zinc applied plants than control. The total chlorophyll, total phenol, total soluble protein, SOD, POD and PAL activity were significant and negatively correlated with WBPH population, whereas, total free amino acid, total soluble sugar and proline content were positively correlated to WBPH population. Protein profiling studies revealed that various combined application of ZnSO<sub>4</sub> and Zn EDTA produced specific polypeptide bands of 66.0 kDa, 37.0 kDa and 14.3 kDa, which was not seen in control. Since, the polypeptide band 23.6 kDa was synthesized in only T<sub>6</sub> (Zn EDTA basal @ 40 kg/ha + Zn EDTA foliar spray @ 0.8 % twice at 30 and 45 DAT) and T<sub>7</sub> (ZnSO<sub>4</sub> basal @ 25kg/ha + Zn EDTA foliar spray @ 0.8% twice at 30 and 45 DAT) which harboured lowest WBPH population, the above protein i.e. 23.6 kDa was considered as most defensive protein responsible for induction of defense mechanism in rice plant to WBPH. Reduction in incidence of WBPH population may be regarded as induced antixenosis, antibiosis and tolerance mechanism in rice plants due to zinc application. It can be inferred that Zn has produced some marked effect in host physiology probably through induction of various metabolites. Therefore, the potential of this compound (zinc) should be exploited in the rice ecosystem and its application can be an eco-holistic approach for effective integration into the pest management system and need to be popularized.

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## APPENDIX-I

**Meteorological data for *kharif*, 2016 (Central Research Farm, OUAT)**

Month	SW	Temperature (°C)			Relative humidity (%)			Rain fall (mm)
		Max.	Min.	Mean	7 hr	14 hr	Mean	
July	27	31.8	26.2	29.00	91	80	85.5	56.4
	28	31.3	25.7	28.50	92	80	86.0	71.0
	29	33.1	25.8	29.45	91	76	83.5	16.0
	30	31.8	25.6	28.70	93	82	87.5	67.6
Aug	31	32.9	25.3	29.10	93	80	86.5	84.5
	32	29.2	25.1	27.15	97	87	92.0	77.0
	33	31.3	25.1	28.20	93	79	86.0	38.7
	34	33.2	26.0	29.60	92	72	82.0	25.0
	35	33.3	26.0	29.65	90	75	82.5	48.5
Sept	36	29.3	24.6	26.95	94	86	90.0	56.5
	37	32.1	25.9	29.00	93	75	84.0	43.8
	38	33.0	26.2	29.60	90	70	80.0	11.6
	39	31.4	25.1	28.25	95	88	91.5	99.7
Oct	40	32.0	24.8	28.40	94	74	84.0	45.1
	41	31.4	24.3	27.85	94	75	84.5	82.8
	42	32.6	21.5	27.05	84	64	74.0	0.0
	43	32.7	20.1	26.40	86	61	73.5	14.0
	44	32.4	22.9	27.65	88	66	77.0	2.8
Nov	45	29.5	17.7	23.60	92	57	74.5	20.3
	46	30.8	18.3	24.55	93	47	70.0	0.0
	47	31.2	15.3	23.25	88	38	63.0	0.0
	48	31.5	16.1	23.80	95	37	66.0	0.0

**Meteorological data for summer, 2016-17 (Central Research Farm, OUAT)**

Month	SW	Temperature ( °C)			Relative humidity (%)			Rain fall (mm)
		Max.	Min.	Mean	7 hr	14 hr	Mean	
<b>Dec</b>	49	30.6	17.2	23.90	82	45	63.5	0.0
	50	30.8	16.2	23.50	79	37	58.0	0.0
	51	29.9	12.8	21.35	90	32	61.0	0.0
	52	29.5	14.4	21.95	94	39	66.5	0.0
<b>Jan</b>	1	29.2	16.0	22.60	96	46	71.0	0.0
	2	29.5	14.6	22.05	83	38	60.5	0.0
	3	29.2	12.7	20.95	86	33	59.5	0.0
	4	30.7	14.1	22.40	91	36	63.5	0.0
<b>Feb</b>	5	31.1	17.6	24.35	95	40	67.5	0.0
	6	33.3	17.2	25.25	94	32	63.0	0.0
	7	33.6	17.4	25.5	92	36	64.0	0.0
	8	35.1	22.4	28.75	92	42	67.0	0.0
<b>Mar</b>	9	35.7	20.6	28.15	94	35	64.5	0.0
	10	33.2	22.6	27.90	93	54	73.5	45.4
	11	33.1	21.6	27.35	92	40	66.0	0.0
	12	35.9	22.7	29.30	89	37	63.0	0.0
<b>Apr</b>	13	35.6	25.3	30.45	88	44	66.0	0.0
	14	35.6	26.0	30.80	88	48	68.0	0.0
	15	37.8	26.0	31.90	86	43	64.5	0.0
	16	36.0	25.9	30.95	88	58	73.0	29.2
<b>May</b>	17	38.3	27.1	32.70	88	49	68.5	0.0
	18	38.0	26.8	32.40	83	47	65.0	0.0
	19	37.9	26.2	32.05	85	45	65.0	27.5
	20	39.4	28.1	33.75	83	46	64.5	0.0
	21	39.2	27.8	33.50	80	45	62.5	0.0

**Meteorological data for *kharif*, 2017 (Central Research Farm, OUAT)**

Month	SW	Temperature (°C)			Relative humidity (%)			Rain fall (mm)
		Max.	Min.	Mean	7 hr	14 hr	Mean	
July	27	31.3	25.8	28.55	90	75	82.5	46.5
	28	32.0	25.4	28.70	94	76	85.0	145.4
	29	31.7	26.1	28.90	93	85	89.0	164.3
	30	31.8	26.3	29.05	92	78	85.0	34.3
Aug	31	33.8	26.1	29.95	91	71	81.0	56.0
	32	33.1	25.6	29.35	90	76	83.0	85.1
	33	32.4	25.8	29.10	93	84	88.5	76.9
	34	33.6	26.0	29.80	87	71	79.0	63.2
	35	32.1	25.3	28.70	96	78	87.0	161.4
Sept	36	33.6	26.0	29.80	92	74	83.0	24.9
	37	34.4	25.7	30.05	90	65	77.5	77.9
	38	32.6	25.8	29.20	92	71	81.5	44.9
	39	33.7	25.5	29.60	92	69	80.5	33.7
Oct	40	30.7	25.1	27.90	94	80	87.0	77.7
	41	33.3	25.4	29.35	94	64	79.0	24.6
	42	31.5	24.8	28.15	93	72	82.5	102.2
	43	33.5	23.2	28.35	92	63	77.5	0.0
	44	31.1	20.6	25.85	90	62	76.0	0.0
Nov	45	31.4	19.5	25.45	86	52	69.0	0.0
	46	27.3	20.7	24.00	87	66	76.5	55.2
	47	29.6	18.7	24.15	91	56	73.5	0.0
	48	29.4	13.9	21.65	92	40	66.0	0.0