“EFFICACY OF KRESOXIM-METHYL TO INDUCE PLANT GROWTH PROMOTING EFFECTS ON CHICKPEA AND WHEAT”

M. Sc. (Ag.) Thesis

by

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“EFFICACY OF KRESOXIM-METHYL TO INDUCE PLANT GROWTH PROMOTING EFFECTS ON CHICKPEA AND WHEAT”

Thesis

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by

Nisha Thakur

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CERTIFICATE –I

This is to certify that the thesis Viva-voce in respect of Miss **Nisha Thakur, a** student of **M. Sc. (Ag.) Department of Plant Pathology** has been conducted under the Chairmanship of Head of the Department along with Advisory Committee on 18/09/2020 The necessary corrections have also been made as per comments/suggestions made by the Advisory Committee and Head of the Department.

Date: 06-10-20

Signature of Major Advisor

CERTIFICATE- II

This is to certify that the thesis entitled **"Efficacy of kresoxim-methyl to induce plant growth promoting effects on chickpea and wheat"** submitted by **Nisha Thakur** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** in the **Department of Plant Pathology** has been approved by the external evaluator and Student's Advisory Committee after oral examination, under the Chairmanship of Head of the Department.

Signature of Head of the Department

Date:- 06-10-20

Major Advisor _____

Faculty Dean _____

Approved/Not approved

Director of Instructions _____

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Date 04-09-20

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LIST OF NOTATIONS AND ABBREVIATIONS

ACC	1-Aminocyclopropane1-Carboxylate
@	At the rate of
C.G,	Chhattisgarh
cm	Centimeter
DAS	Days after sowing
^{o}C	Degree Celsius
et al.	And other
E.D.T.A	Ethylenediaminetetraacetic acid
WUE	Water Use Efficiency
GLAD	Green Leaf Area Duration
g	Gram
hrs	Hours
i.e.	That it
kg	Kilogram
MDA	Malondialdehyde
ml	Milliliter
mm	Millimeter
NBT	Nitroblue Tetrazolium
No.	Number
NUV	Near Ultra Violets
pH	Potential of Hydrogen
ppm	parts per million
rpm	Revolutions Per Minute
SDW	Sterilized Distilled Water
SC	Suspension concentrate
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
UV	Ultra Violets
Wt.	Weight
viz.	Namely

THESIS ABSTRACT

Title of the thesis	:**"Efficacy of kresoxim-methyl to induce plant growth promoting effects on chickpea and wheat"**
Full name of the student	: Nisha Thakur
Major subject	: Plant Pathology
Name and Address of Major Advisor	: Dr. Anil. S.Kothesthane, Head and Professor, Dept. of Plant Pathology, CoA, IGKV, Raipur.
Degree to be Awarded	: M. Sc (Ag.) Plant Pathology

Signature of Student

Signature of Major Advisor

Date: 04-09-20

Signature of Head of the Department

ABSTRACT

The strobilurins belong to an important new fungicide class, with a distinct mode of action that targets fungal mitochondrial respiration. Kresoxim-methyl is effective at low concentrations and protects many plant pathogenic fungi, its primary function is disease management. Kresoxim-methyl decreases primary infection and pathogen spread by strongly inhibiting spore germination, so post-infection applications against powdery mildews and apple scab can control advanced infections, symptom transmission, and sporulation, thus preventing further development of the disease. Such properties make kresoxim-methyl an efficient compound against these pathogens in IPM programs. Studies also suggest that kresoxim-methyl has shown that strobilurins can have important effects on metabolism in plants. It has been reported to improve photosynthetic efficiency, influence the hormonal status of the plant through a bioregulatory auxin-like activity. The present investigation was therefore undertaken to investigate the effects of Kresoxim-methyl three crops Wheat, Rice, and Chickpea. Experiments

were conducted under field and also under disease-free conditions in the rainout shelters.

Experiments were conducted to find the effect of four doses of Kresoxim-methyl on germination, and radicle morphology of wheat, rice, and chickpea. Growth promoting effects were observed on germination and radicle morphology of these crops prompted the author in support of the argument that kresoxim-methyl displays a similar mode of action to that of plant growth-promoting bacteria. Will the combined application of fluorescent *Pseudomonas* and kresoxim-methyl have a significant positive effect on plant metabolism resulting in growth and yield in different crops? The dual effect of fluorescent *Pseudomonas* applied as seed treatment and kresoxim-methyl as a spray during the subsequent growth stages of the crop on plant growth stimulation was also studied. Two fluorescent *Pseudomonas isolates* 9704 and 9829 were used. Wheat, rice, and chickpea were sprayed with four doses of Kresoxim-methyl, and observations were recorded on different plant morphological parameters. Differences in radical length and frequency of root hairs and lateral roots in germinated seedlings were observed in kresoxim-methyl treatments. Foliar applications of Kresoxim-methyl significantly improved morphological traits (root and shoot) in healthy wheat, rice, and chickpea plants. Variations in the levels of different enzymes analyzed were inconsistent and therefore it could not be conclusively demonstrated that Kresoxim-methyl modifies the levels of different enzymes in these crops.

शोध सारांश

शोध का शीर्षक : "चने और गेहूं के पौधों के विकास को प्रेरित करने के लिए क्रेसॉक्सिम मिथाइल की प्रभावकारिता"

छात्रा का पूरा नाम : निशा ठाकुर

प्रमुख विषय : पौध रोग विज्ञान

प्रमुख सलाहकार का नाम और पता : डॉ. ए.एस. कोटस्थाने, प्रोफेसर एवं प्रमुख, (पादप रोग विज्ञान विभाग), कृषि महाविद्यालय, रायपुर

उपाधि का नाम : एम.एस.सी. (कृषि) पौध रोगविज्ञान

छात्रा का हस्ताक्षर

प्रमुख सलाहकार का हस्ताक्षर

दिनांक: : 04-09-20

विभाग के प्रमुख का हस्ताक्षर

सारांश

स्ट्रोबिल्यूरिन एक महत्वपूर्ण नए कवकनाशी वर्ग से संबंधित है, जिसका कार्य करने का एक अलग तरीका है जो कवक के माइटोकॉन्ड्रियल श्वसन को लक्षित करता है कम सांद्रता पर क्रेसॉक्सिम.मिथाइल प्रभावशाली पाय और कई पौधों को रोगजनक कवको से बचाता हैं जो कि रोग प्रबंधन में इसका प्राथमिक कार्य है। क्रेसॉक्सिम .मिथाइल प्राथमिक संक्रमण को कम कर देता है और दृढ़ता से कवक बीजाणु अंकुरण को रोकता है, इसलिए पाउडरी मिल्ड्य एवं सेब स्केब के संक्रमण के बाद प्रयोग कर संक्रमण को नियंत्रित कर सकते हैं, लक्षण संचरण एवं स्पोरुलेशन, इस प्रकार रोग के आगे विकास को रोका जा सकता हैं । इस तरह के गुण आईपीएम कार्यक्रमों में इन रोगजनकों के खिलाफ क्रेसॉक्सिम.मिथाइल को एक प्रभावशाली यौगिक बनाते हैं ।

अध्ययन यह भी सुझाव देते हैं कि क्रेसॉक्सिम.मिथाइल से पता चला है कि स्ट्रोबिल्यूरिन्सन का पौधों में चयापचय पर महत्वपूर्ण प्रभाव पड़ता है । यह प्रकाश संश्लेषक दक्षता में सुधार करने के लिए भी पायण गया हैए जैव नियामक ऑक्सिन जैसी गतिविधि के माध्यम से पौधे की हार्मोनल स्थिति को प्रभावित करता है । वर्तमान जांच मे क्रेसॉक्सिम.मिथाइल तीन फसलों गेहूं, चावल और चना के प्रभाव की जांच करने के लिए किया गया । यह प्रयोग बारिश के आश्रय क्षेत्र के अंतर्गत और रोग.मुक्त परिस्थितियों में भी प्रयोग किए गए । अंकुरण पर क्रेसॉक्सिम.मिथाइल की चार बढ़ती हुई खुराक और गेहूंए चावल और चना के मूल आकारिकी के प्रभाव को देखने के लिए प्रयोग किया गया । गेहूं , चावल और चना के अंकुरण और मूलाधार आकृति विज्ञान पर देखने पर विकास को बढ़ावा देने वाले प्रभावों ने भी प्रेरित किया, क्योंकि क्रेसोक्सिम. मिथाइल पौधे के विकास को बढ़ावा देने वाले जीवाणुओं के लिए एक समान तरीका प्रदर्शित करता है। क्या ;फ्लोरेसेंट स्यूडोमोनस और क्रेसोक्सिम.मिथाइल, के संयोजन का उपयोग पौधे के चयापचय पर महत्वपूर्ण सकारात्मक प्रभाव पड़ेगा, जिसके परिणामस्वरूप विभिन्न फसलों में वृद्धि और उपज होती हैद्ध हमने पादप विकास उत्तेजना पर फसल के विकास चरणों के दौरान फुहार के रूप में बीज उपचार और क्रेसॉक्सिम.मिथाइल के माध्यम से लागू फ्लोरोसेंट स्यूडोमोनस के दोहरे प्रभाव की जांच की। दो फ्लोरोसेंट स्यूडोमोनस 9704 और 9829 अलग.थलग थे। गेहूंए चावल और चना को क्रेसोक्सिम.मिथाइल की चार बढ़ती खुराक के साथ छिड़का गया और विभिन्न पौधों के रूपात्मक मापदंडों पर अवलोकन दर्ज किए गए। क्रेसॉक्सिम मिथाइल ट्रीटमेंट में अंकुरित अंकुरों के मूल में लंबाई और पार्श्व बालों की जड़ों और पार्श्व जड़ों में अंतर बढ़ गया। रोग मुक्त गेहूँ, चावल और चना के पौधों में क्रेसोक्सिम.मिथाइल के फोलियर अनुप्रयोग करने पर रूपात्मक लक्षणों ;जड़ और शूट, में काफी सुधार पाया गया । वर्तमान जांच में विश्लेषण किए गए विभिन्न एंजाइमों के स्तरों में भिन्नता असंगत थी और इसलिए हम निर्णायक रूप से यह प्रदर्शित नहीं कर सके कि क्रेसोक्सिम.मिथाइल विभिन्न एंजाइमों के स्तर को संशोधित करता है।

CHAPTER- I
INTRODUCTION

Agriculture is a major sector in meeting the food and nutritional security of the global population. India ranks second with a share of 8% in agricultural output. India's agricultural production in the year 2014 was $367 billion. Indian agriculture at present is functionally distinct and vigorous than the one prevalent during the time of Green Revolution which began in 1970s from 1970s until the late 1990s, During almost three decades, India's agricultural GDP expanded slowly from $25 billion to $101 billion as the GDP was centered mainly to rice and wheat.

Agriculture plays an important part in the Indian economy. Agriculture is an important sector of the Indian economy, contributing about 17% to the overall GDP and providing jobs to over 60 % of the population. India's economy depends mostly on agriculture and at present, it is among the top two farm producers in the world. India is rising economically but the profit of this progress is mostly restricted to urban or semi-urban areas but as we know approximately 65% of the population lives in rural areas and depends on agriculture and related avenues for their livelihood. Agriculture accounts for over 70 percent of rural households. At least two-thirds of the working population are dependent on agriculture to earn their living. 15-25 percent of potential crop production is lost due biotic and abiotic stress, at a time when India needs to raise the production and also to ensure nutrition and food security for its growing needs of consumption. With the increasing population and rise in their demands, the need of protecting food crops from diseases and pests have become challenging. The loss in crop yield due to weeds, disease, and pests, which are of eminent importance, and loss approximately ranging between 10 to 30% of crop productions. An average of 20% crop loss leads to lose Rs. 1,40,000 crores, which is enormous (Kumar and Gupta, 2012).

Acknowledging the ever-increasing requirement for food production with the inadequate availability of irrigation water and the shrinking cultivable area, it is needed to go for agro-chemicals for production and protection of crops. But, the use of various chemicals indiscriminately will bring down soil fertility, increase the toxicity, pest and disease resistance, labor cost, and also will result in health and environmental hazards. Hence, advocacy of modern agrochemicals which can heighten the growth and yield of crops, in addition to pest and disease control is necessary to overcome these problems.

Due to plant disease, almost 20% of yield reduction globally can be seen in major food and cash crop and fungicides act as an essential solution for the effective control of plant diseases. Fungicides are known to produce fungicidal effects on fungal pathogens. However, it has been reported that some pesticides may have significant adverse effect on plant physiology. And some fungicide like strobilurins have been reported to induce physiological changes in crops. The strobilurins form a distinct class of fungicidal compounds which can be isolated from several species of Basidiomycete which inhabit decaying plant material in woodland soils. Original isolations of strobilurin A and B were made from *Strobilurus tenacellus*. It has been documented that strobilurins cause physiological changes in crops by increasing tolerance to abiotic stress, appearance of darker green leaves, delay in maturity of photosynthetic leaf area, maintenance of the balance of phytohormones, and increase uptake of carbon dioxide. Many fungicides which once controlled the pathogen developed resistance towards the organisms due to the fact that fungicides were having single-site inhibitors of fungal metabolism. A derivative of fungal secondary metabolite strobilurin A, kresoxim-methyl inhibits mitochondrial respiration (Sauter *et al*., 1995; Anke, 1997). Strobilurin's fungicidal mode of action is the inhibition of fungal mitochondrial respiration by binding to a particular cytochrome b site (the ubiquinol site), which inhibits the transfer of electrons between cytochrome b and cytochrome c (Ammermann *et al*., 1992; Earley *et al*., 2012). By inhibiting ubiquinol-cytochrome c-oxide reductase, they disturb the respiratory process which blocks the energy supply (ATP) of the fungus cell. As a result, the mitochondria

stop producing energy and ultimately the fungus die. They are also known as QoIs or bc1 complex inhibitors (Sauter *et al.*, 1999; Bartlett *et al.*, 2002; Balba 2007).

Family Strobilurin consists of ubiquinol oxidase inhibitor (Qol) known to enhance certain morphological attributes of *Zea mays* (maize), such as an increase in number of leaf and its area, and increase in shoot and root biomass (Lazo and Ascencio, 2014). Strobilurins increases the tolerance to abiotic stress, such as delay in senescence of the photosynthetic leaf, maintain the amount of the phytohormones, and increase absorption of carbon dioxide in wheat (Köhle *et al*., 2002). It was also found to induce non-fungicidal physiological changes in wheat (Grossmann and Retzlaff, 1997) like appearance of darker green, delayed maturity, increased stress tolerance and favoured plant biomass and corn production (Grossmann and Retzlaff, 1997). The strobilurin based kresoxim-methyl provides an essential resistance management tool because its efficacy against target pathogens is not affected by the occurrence of strains resistant to other fungicides (Ypema *et al.,* 1999). One of the most positive side effects reported by Strobilurins (Qo inhibitors) is that it results in an increase in kernel weight grain yield and contents of protein with a delay in maturity. It also has been reported to improve efficiency of photosynthesis, influences the hormonal status of the plant through a bioregulatory auxin-like activity.

The fungicide class azole also affects the physiology of plants treated with it as in case of winter wheat by rise in the chlorophyll content, delay in leaf maturity, and also protects them from abiotic stresses (Fletcher *et al*., 2010). Movement of sedaxane from the treated seed to the rhizosphere of soil and enters inside the plant tissues which is found to initiate the root and shoot development of cereals (Swart, 2011). Previously it has been described that wheat responds to sedaxane positively in terms of increase in biomass and growth and resistance to drought (Ajigboye *et al*., 2016).

Application of kresoxim-methyl led to decrease in the level of 1-aminocyclopropane1-carboxylic acid (ACC), an ethylene precursor, and ACC synthase activity and subsequently decrease in ethylene production. Lower ethylene concentrations have been shown to slow degradation of cytokinins

(Bollmark, & Eliasson, 1990), which have been shown to delay senescence (Badenoch-Jones *et al*., 1996), and increase chlorophyll production (Kuroda *et al.*, 1996). The result of reduced chlorophyll degradation, greater assimilate concentrations, and delayed senescence is seen when plants are treated with kresoxim-methyl and it led to increase in growth, yield and photosynthesis rate.

In the view of above points the present studies will be carried out in Department of Plant Pathology and Department of Plant Molecular Biology and Biotechnology, College of Agriculture I. G. K. V, Raipur with the following objectives**: -**

1. To study plant growth promoting effect of spray application of kresoxim-methyl and seed treatment with fluorescent *Pseudomonas* on chickpea and wheat plants.
2. Assessment of water stress tolerance in wheat plants derived from spray application with kresoxim-methyl and seed treatment with fluorescent *Pseudomonas.*
3. Assessment of anti-oxidant enzyme in chickpea plants derived spray application with kresoxim-methyl and seed treatment with fluorescent *Pseudomonas.*

CHAPTER- II
REVIEW OF LITERATURE

Fungicides are known to have fungicidal effects on the pathogenic fungi. Strobilurins have been reported to cause physiological changes in crops by increasing abiotic stress tolerance, darker green appearance of leaves, delayed senescence ofleaf area, maintained phytohormonal balance, and increased carbon dioxide uptake. The strobilurins form a distinct class of fungicidal compounds which originated from several species of Basidiomycete which inhabit decaying plant material in woodland soils. Initially isolations of strobilurin A and B were made from *Strobilurus tenacellus*. It has been documented that strobilurins may cause physiological changes in crops by increasing tolerance to abiotic stresses, appearance of darker green leaves, delayedmaturity of photosynthetic leaf area, maintenaned balance of phytohormones, and increased uptake of carbon dioxide. The many fungicidal resistance is reported in pathogens in case of fungicides which have single-site inhibitors of fungal metabolism. A derivative of fungal secondary metabolite strobilurin A, kresoxim-methyl inhibits mitochondrial respiration. Strobilurin's fungicidal mode of action is the inhibition of fungal mitochondrial respiration by binding to a particular cytochrome b site (the ubiquinol site), which inhibits the transfer of electrons between cytochrome b and cytochrome c. By inhibiting ubiquinol-cytochrome c-oxide reductase, they disturb the process of respiration which blocks the energy supply (ATP) of the fungus cell. As a result, the mitochondria stop producing energy and ultimately the fungus dies. They are also known as QoIs or bc1 complex inhibitors.

Strobilurins (Qo inhibitors) results showed increase yields, weight of kernel, and protein content correlated with a delay in senescence of the flag leaf. One of the most positive side effects recorded is the strobilurin-induced “Green effect”. Sauter *et al.*,(1995) stated mitochondrial respiration is inhibited by which is a derivative of fungal secondary metabolite strobilurin A.

Grossmann and Retzlaff, (1997) also reported kresoxim-methyl induce non-fungicidal bioregulatory changes in the wheat. Strobilurins were described to cause bioregulatory changes in crops through increased tolerance to abiotic stress, the appearance of darker green leaves, delayed photosynthetic leaf area maturity, and favoured corn production and plant biomass. Kresoxim-methyl has been reported to boost photosynthetic efficiency by influencing the plant hormonal status through a bio-regulatory auxin-like action.In addition to its fungicidal influence, strobilurin kresoxim-methyl has been reported to induce physiological and developmental changes in wheat (*Triticum aestivum* L.) as seen in relation with enhanced yield.

2.1 The bioregulatory effect of fungicide kresoxim methyl on plant physiology.

2.1.1 Mode of action

Bartlett *et al.,* (2002) reported various synthetic fungicides such as kresoxim-methyl methyl (E)-α(methoxyimino)-2-(2-methylphenoxymethyl) phenylacetate that was a modified form of the naturally occurred strobilurin A compound. The strobilurins bind to one particular site of the mitochondria, the quinol oxidation (Qo) site (or ubiquinol site) of cytochrome b and thereby preventing the transfer of electrons between cytochrome c and cytochrome b, which stops the adenosine triphosphate synthesis and nicotinamide adenine dinucleotide (NADH) oxidation. This cessation of energy production ultimately leads to death of fungi. Their mode of action is therefore novel and new target-specific.

Sauter *et al.,* (1995) stated mitochondrial respiration is inhibited by kresoxim-methyl which is a derivative of fungal secondary metabolite strobilurin A. A short-term effect on plant mitochondria does not necessarily resulting phytotoxicity because the toxicity at organism-level is determined by the importance of mitochondrial respiration for energy supply, which varied with the organism's environmental conditions and life-stage.

Ammermann *et al.,* (1992) reported that a new fungicide Kresoxim-methyl is derived from the fungal secondary metabolite strobilurin A, which acts by blocking the transfer of fungal electrons to the mitochondrial respiration cytochrome-bc complex.

2.1.2 Harmonal regulation

Taize Zeiger, (2004) reported that the biosynthesis of ethylene can be induced by any type of lesion, which includes physiological stress caused by water stress, cooling, temperature, diseases, or inundation. In stress reactions, ethylene acts as the primary hormonal mediator of plant senescence.

Grossmann and Retzlaff, (1997) reported kresoxim-methylase a hormonal regulator or hormone like compound. A variety of bio tests, which included isolated mustard shoots and heterotrophic maize, germinating cress seeds, duckweed, and photoautotrophic algal cell suspensions. They showed a similar pattern of reaction to standard auxins (e.g. indol-3-acetic acid, IAA; 2-(1-naphthyl) acetic acid, a-NAA). When in hormone-free medium stem explants of tobacco were cultured, auxin-like activity of kresoxim-methyl was also found. The most notable changes were the reductions in the levels of ACC and ethylene formation shown in intact plants, leaf discs and wheat shoots under drought stress. Kresoxim-methyl affected the induction of ACC synthase activity which changes S-adenosyl-methionine to ACC in ethylene biosynthesis.

Abeles *et al.*, (1992) worked in cultures such as wheat, stress by ethylene impairs production by the beginning of premature ripening of the grains and promoting leaf senescence, which reduces the production of assimilates and the period of grain filling. Application of kresoxim-methyl led to a decrease in the level of 1- aminocyclopropane1-carboxylic acid (ACC), an ethylene precursor, and ACC synthase activity and subsequently decrease in ethylene production.

2.1.3 Physiological parameters

Amaro *et al.*, (2018) worked to assess the effects on grafted and ungrafted Japanese cucumber (*Cucumis sativus* L.) on the application of pyraclostrobin, boscalid and azoxystrobin to investigate the fungicidal effects on physiology of the plant and increased fruit production. The grafted plants treated with pyraclostrobin or boscalid alone, the physiological benefits were more apparent and these benefits manifested in terms of fruit production and increased the function of the antioxidant system, thus reducing stress.

Dingenen *et al.,* (2017) analyzed effect of strobilurin kresoxim-methyl on Arabidopsis leaf growth. Seedlings treatment with Stroby resulted in broader leaves because of an increase in number of cells. It was reported to have a growth-enhancement impact of sedaxane on wheat. The experiments examined the possible impact of bio stimulating and related bioregulatory changes in maize (*Zea mays*) seedlings maintained under sterilized conditions when treated with increasing doses of sedaxane under controlled condition. They stated it has significant auxin-like and gibberellin-like effects, which effect marked morphological and physiological changes.

Filippou *et al.,* (2015), conducted an experiment on pre-treatment of Kresoxim-methyl in *Medicago truncatula* plants, resultant improved defence against drought and salt stress. Foliar application of Kresoxim-methyl before the stress imposition resulted in improvement of physiological parameters compared with stressed-only plants. This protective effect was further assisted by increased biosynthesis of the proline, modified signalling of reactive oxygen and nitrogen compounds, and cellular damage reduction.

Ajigboye *et al.,* (2014) evaluated the impact of application of certain fungicides, including azoxystrobin, isopyrazam, and epoxiconazole, on the biomass, production, and yields of Photosystem II (PSII) of the winter wheat. They concluded that applying a particular combination of fungicides in the absence of pressure from the disease resultant in increased yield and biomass in winter wheat.

Solorzano and Malvick, (2011) conducted an experiment in growth chamber, laboratory and field, to analyze the impact of seed treatment with different fungicides such as fludioxonil, azoxystrobin and mefenoxam, on germination, grain yield and plant populations, of low-quality hybrid maize seed which was infected with seedborne fungal pathogens. They reported increase germination, yield and population, of maize seed treated with fungicide azoxystrobin and fludioxonil.

Bertelsen *et al.,* (2001), performed an experiment in which fungicide azoxystrobin's impact on yield and senescence in winter wheat were examined. Experiment was performed in field trials which were free of

apparent disease and under controlled conditions. Cereals crops with application of strobilurin had higher yields and better-quality cereals. In winter wheat, the effects of azoxystrobin (a strobilurin) and epoxiconazole fungicides (a sterol biosynthesis inhibitor) on phyllosphere fungi, senescence and yields were investigated. Treatments with each of the two fungicides prolonged green leaf area retention in two field trials and increased yield compared to untreated control plots.

Grossmann, Kwiatkowski and Caspar, (1997) used leaf discs and intact wheat plants (Triticum aestivum L.) to research the physiological effects of kresoxim-methyl strobilurin-type fungicide in relation to induced phytohormonal changes. Dose-response experiments showed that kresoxim-methyl modified the hormonal balance, preferring dihydrozeatin riboside-type cytokinins as compared to ethylene and its 1-aminocyclopropane-1-carboxylic acid (ACC) biosynthetic precursor. This was closely associated with delayed senescence of the leaf. Kresoxim-methyl was demonstrated to inhibit ACC synthase induction in the formation of ethylene. In addition, kresoxim-methyl caused the endogenous levels of abscisic acid, relative to the regulation, to increase up to two-fold. Concomitantly, plants stomatal aperture and water intake were decreased. Kresoxim-methyl is suggested to change the hormonal configuration in wheat which results in delayed senescence of the leaf and water-conserving effects.

Grossmann and Retzlaff, (1997) conducted a variety of biotests on isolated shoots of mustard and heterotrophic maize, germinating cress seeds, duckweed, and photoautotrophic algal cell suspensions. They represent a similar pattern of reaction to standard auxins (e.g. indol-3-acetic acid, IAA; 2-(1-naphthyl) acetic acid, a-NAA). When in hormone-free medium stem explants of tobacco were cultured, auxin-like activity of kresoxim-methyl was also found. The most notable changes were the reductions in the levels of 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene formation under drought stress shown in intact plants, leaf discs and wheat shoots. Kresoxim-methyl affected the induction of ACC synthase activity which converts S-adenosyl-methionine to ACC in ethylene biosynthesis.

Kresoxim-methyl, delayed leaf senescence, characterised by decreases loss of chlorophyll, when leaf discs were incubated in the dark for three days. An AVG that effectively inhibited the formation of ethylene also delayed the senescence of the leaf. This suggests the ethylene synthesis inhibition have been induced by kresoxim-methyl appears to be linked to its role of delaying the senescence. The remarkable physiological effects of Strobilurin fungicides on the crop yields, due to activity of the enzyme nitrate reductase, stress tolerance, the promotion of net carbon assimilation and hormonal balance. When added alternately or together, the carboxamides reinforce the action of strobilurins.

2.2 Management of water stress by fungicide kresoxim methyl

Giuliani *et al.*,(2018) conducted an experiment with the objective of assessing the combined effect of deficit irrigation and treatment of fungicide strobilurin in two genotypes of tomato. Water use efficiency (WUE), physiological, yield and quality parameters and expression of ERD15, a gene involved in the response to abiotic stress, were evaluated. Strobilurin dictated lower conductivity of stomata, maintaining a higher assimilation rate, resulting in an increase in WUE.

Filippou *et al.*, (2015) conducted a study to investigate kresoxim-methyl primes on *Medicago truncatula* plants against abiotic stress factors. Kresoxim-methyl is a proven chemical agent commonly used as a strobilurin fungicide, now emerged as a novel priming inducer, combined with differential protection against two abiotic stress factors, which are salinity and drought. Foliar application of Kresoxim-methyl in *M. truncatula* plant significantly improved the adverse effects on salinity and drought on plant physiology, supporting stress control modulation by biochemical and molecular processes and regulation of a multitude of cell. By regulating plant metabolism, kresoxim-methyl acts as a protective molecule and sustains the abiotic stressed response mechanism. The metabolism of *M. truncatula* has been shown to be able to resolve stress-induced oxidative consequences following pretreatment with, kresoxim-methyl by controlling independent pathway-specific processes.

Berdugo *et al.*, (2012) conducted a study to evaluate the effect on senescence and yield formation of wheat treated with bixafen. Bixafen, a pyrazole carboxamide inhibiting fungal respiratory chain succinate dehydrogenase, is a new broad-spectrum fungicide developed for the control of cereal pathogens. Treatment with prothioconazole, fluoxastrobin, and bixafen, also delayed the leaf senescence. When it was compared to untreated and spiroxamine treated plants there was significantly increased the duration of the green leaf. Differences in leaf and ear senescence between treatments were confirmed as an indicator of transpiration activity by measuring the temperature of the wheat tissue. All the treatments with fungicides increased grain yield.

Wu and Tiedemann, (2001) conducted an experiment to study the physiological effects of epoxiconazole and azoxystrobinon the senescence and the oxidative status of wheat.The senescence process has been well defined by the decrease in the total protein content of leaves and the increase in leakage of electrolytes from leaf tissue. The increases in these two senescence factors associated with a rise in superoxide levels and reduced antioxidant enzyme superoxide dismutase (SOD) activity during senescence. The senescence was significantly delayed by the use of azoxystrobin and epoxiconazole, resulting in an increase in total SOD activity, especially at mature growth stages.

Grossmann and Retzlaff, (1997) demonstrated that by reducing the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, strobilurin kresoxim-methyl suppresses ethylene biosynthesis in the wheat bud tissue. This was due to the delay in the senescence of the leaf, resulting in prolonged green tissue photosynthetic activity and improved stress management. Ethylene can be produced in almost all parts of plant tissue, although the production rate depends on the stage of development and type of tissue.

2.3 Antioxidant Enzyme Assay

2.3.1 Superoxide dismutase (SODs)

Koehle *et al.* (1997) reported that leaf SOD activity is the primary scavenger for superoxide radicals and decreases in ageing leaf tissue,

consistent with increased development of reactive intermediate oxygen (ROI) during plant senescence. Pyraclostrobin treatment improved the scavenging of SOD activity in barley leaves by superoxide.

Larson, (1997) reported that anti-oxidant enzymes, like superoxide dismutase (SOD), catalase, and peroxidase, are released in oxidative stress plants. Unfavorable atmosphere (stressors) promotes radical formation, in specific types of reactive oxygen, and increases the oxidative capacity of plant tissues.

Beauchamp and Fridovich, (1971) described a method in which all extracts were assayed for SOD activity photochemically, using the assay system consisting of methionine, riboflavin, and NBT.

McCord and Fridovich, (1969) first described this enzyme activity with a cupro-zinc protein (erythrocuprein) from bovine erythrocytes. Superoxide dismutases are metalloproteins that catalyzes the dismutation of the superoxide free radical (0_2^-) to molecular oxygen and H_20_2.

2.3.2 Phenylalanine ammonia lyase (PAL)

Halbrook and Sheel, (1989) stated phenylalanine ammonia lyase as an antioxidant, catalysing the determination of L-phenylalanine to produce cinnamic acid; a substratum feeding many biosynthetic routes to different groups of secondary plant products derived from phenylpropanoids.

Brueske, (1980) described a method to measure the phenylalanine ammonia lyase behaviour by measuring the quantities of t-cinnamic acid (t-CA) formed.

2.3.3 Peroxidase

Hammerschmidt *et al.*,(1982) described a method to estimate peroxidase activity (POx) spectrophotometrically. In that method, they inoculated the first true leaf of cucumber with *Colletotrichum lagenarium* to study peroxidase activity in leaf.

2.3.4Total phenolic content (TPC)

Zheng and Shetty (2000) for the purpose of investigating the impact of apple pomace extracts fermented with Trichoderma spp, they performed a pea

study (*Pisum sativum*) on phenolic content and seedling vigour in peas, and identified a method of estimating total phenolic content.

CHAPTER- III
MATERIALS AND METHODS

The present investigation entitled**: "Efficacy of kresoxim-methyl to induce plant growth promoting effects on chickpea and wheat."** was carried out in Department of Plant Pathology and Department of Plant Molecular Biology and Biotechnology, College of Agriculture I.G.K.V, Raipur. Field and pot experiments were carried out at research cum instructional farm, College of Agriculture, I.G.K.V, Raipur.

Materials used and methodology adopted for the study is described in this chapter.

3.1 Materials

3.1.1 Fungicide Kresoxim-methyl

Fungicide Kresoxim-methyl was tenderly provided by Dr. Anil. S. Kotasthane, Professor and Head of Department of Plant Pathology, CoA, IGKV, Raipur. The four different concentration of fungicide Kresoxim-methyl were tested for growth and yield attributing parameters following spray application in pot and field experiments

3.1.2 Fluorescent *Pseudomonas* isolates

Fluorescent *Pseudomonas* isolates used in the present study were tenderly provided by Dr. Anil. S. Kotasthane, Professor and Head of Department of Plant Pathology, CoA, IGKV, Raipur. The Fluorescent*Pseudomonas* isolates were tested for growth and yield attributing parameters following seed treatment in pot and field experiments.

3.1.3 Plant material

Seed of rice (Swarna), chickpea (INDIRA CHANA-1) and wheat (Amber wheat?)were used for pot and field experimentsandweresourced from KVK farm, College of Agriculture, I. G. K. V, Raipur.

3.2 Methods

3.2.1 Assessment of plant growth promoting effect

3.2.1.1 Germination Test

Seed of crops were treated with four doses of Kresoxim-methyl (44.3% SC), 310.17 ppm (14µl/20ml of SDW), 354.48 ppm (16µl/20ml of SDW), 398.29 ppm (18µl/20ml of SDW) and 443ppm (20µl/20ml of SDW). Seed germination test was performed in interfitting plastic petri plate Is it approved method by ISTA?. Two good quality blotter papers of the same diameter were kept and moistened with sterilized distilled water. Lid and bottom of the plates were lined with moistened blotter paper to create moist chamber. In each plate, ten seeds were placed on the moistened blotters in such a manner that nine seeds formed the outer circle and one at the center. For each lot of seed, 3 replicated plates were maintained (total of 30 seeds tested for each lot of seed). Plates were then incubated at 27±1ºC for seven days in alternating cycles of 12 hours'darkness and 12 hours' light in NUV. Observations were recorded on per cent germinated seed. Plants grown from treated seeds were compared with untreated controls.

3.2.1.2 Seed treatment

Seed of rice chickpea and wheat were treated with the potential fluorescent *Pseudomonas* isolates. The potential fluorescent *Pseudomonas* isolates were grown in 100 ml Kings B base broth in 250ml conical flasks on a rotary shaker (150 rpm/min) for 48hrs at room temperature. Culture broth was used as bacterial inoculum. The bacterial suspensions were prepared. Slurry for seed bacterization was prepared by adding bacterial culture and talcum powder to seeds of rice, wheat and chickpea. Wheat seeds were treated with fluorescent *Pseudomonas* isolates 9829 and 9704. Care was taken for ensuring uniform coating of all seeds. Untreated seeds were kept as control.

3.2.1.3 Pot experiment

Seed of rice, and wheat were used for pot experiment. Seed bacterization was done as described in above paragraph. Plants of wheat were grown in cylindrical PVC pots in rainout shelter. The pots were filled with a sterilized mixture (36 h in an oven at 120°C) of silty-loam soil collected from a field on the experimental farm (pH 8.4), and fine sand (1:1 w/w) to facilitate water drainage and root collection, to

which was added a standard dose of pre-sowing fertilizer (about 100 kg N ha^{-1}, 150 kg P_2O_5 ha^{-1} and 300 kg K_2O ha^{-1}). Wheat seeds were treated with fluorescent *Pseudomonas* isolates 9829 and 9704. Seeds treated with potential fluorescent *Pseudomonas* strains were sown at 10 seeds per pot separately. Untreated control pots were maintained for comparative studies. Three replicas were maintained for each treatment. After fifteen days of sowing first fungicidal spray application was done on pot with four increasing doses of Kresoxim-methyl (44.3% SC): 310.17 ppm (14µl/20ml of SDW), 354.48 ppm (16µl/20ml of SDW), 398.29 ppm (18µl/20ml of SDW) and 443 ppm (20µl/20ml of SDW). Plants grown from bacterized (isolates 9829 and 9704) seeds and also untreated seeds were sprayed with four increasing doses of Kresoxim-methyl. Second spray was done after thirty days of first spray with same increasing dose of kresoxim-methyl. Plants grown from untreated treated seeds were compared with untreated controls. Growth measurements were taken from three pots/plants. Observation such as bundle weight, shoot length, root length, plant height, root volume, root weight, yield, etc were recorded during maximum maturity stage of crop.

3.2.1.4 Root scanning

45 days old seedlings were used for root scanning. Roots of rice were uprooted and washed. Care was taken during uprooting and washing of roots to not damage roots. Using root analysis software package WinRHIZO, five roots of respective treatments were analysed. Analysis such as root length, root volume, surface area, number of root tips, number of forks as compared to control were recoeded.

3.2.1.5 Field experiments

Field experiments was conducted at Research cum instructional Farm, College of Agriculture, I. G. K. V, Raipur, to study the efficacy of potential fluorescent *Pseudomonas* isolates on growth and yield attributing characters following seed treatment in chickpea. Chickpea seeds were treated with fluorescent *Pseudomonas* isolates 9829 and 9704. Seed treatment was done by spraying potential *Pseudomonas* isolates on seeds and talcum powder was sprinkled so that bacterial culture could coat the seeds completely and shade dried. Seed treatment was done a day before sowing. Three replications were maintained for each

treatment in randomized block design and untreated control was maintained for comparative purpose. After thirty days of sowing first, fungicidal spray was done with four increasing dose of fungicide kresoxim-methyl (44.3% SC): 310.17 ppm (14µl/20ml of SDW), 354.48 ppm (16µl/20ml of SDW), 398.29 ppm (18µl/20ml of SDW) and 443ppm (20µl/20ml of SDW). Second spray was done after forty-five days of first spray application. Observations such as bundle weight and grain yield were recorded.

3.2.2 Assessment of water stress tolerance in wheat plants

Seeds of wheat were treated with two different isolates of fluorescent *Pseudomonas*. Seed treatment was done by spraying potential *Pseudomonas* isolates 9704 and 9829 on seeds and talcum powder was sprinkled so that bacterial culture could coat the seeds completely and shade dried. Seed treatment was done a day before sowing. Three replications were maintained for each treatment in pots and untreated control was maintained for comparative purpose. After thirty days of sowing fungicidal spray will be done with four increasing dose of fungicide kresoxim-methyl (44.3% SC): 310.17ppm (14µl/20ml of SDW), 354.48 ppm (16µl/20ml of SDW), 398.29 ppm (18µl/20ml of SDW) and 443ppm (20µl/20ml of SDW).The second spray was done after thirty days of first spray. After 15 days the watering will be stopped, which will a create water stressed condition. A comparison will be done among only KM fungicide treated plants, KM+fluorescent *Pseudomonas* treated plants and untreated plants.

3.2.3 Antioxidant enzyme assay

3.2.3.1 Determination of antioxidant activities

Different enzymes were assayed in leaves of chickpea. Seeds treated with different strains of fluorescent *Pseudomonas* during crop maturity stage. For preparation of leaf sample, fresh green flag leaves of chickpea were collected from the field and taken to laboratory in fresh form by keeping the samples in ice box. Leaf samples were frozen in liquid nitrogen in laboratory and grinded to fine powder using fresh pestle and motor and powder was collected in 50 ml tarson tube and stored at -20^{o}C for further analysis.

3.2.3.1.1 Super oxide dismutase activity (SOD) (E.C.1.15.1.1)

SOD activity was measured by the method described by Fridovich., 1974 using riboflavin/methionine system.

Principle

Superoxide radical reduces the NBT resulting in the formation of blue color formazan. NBT method for SOD assay is based on principle that NBT undergo photoreduction (which is a blue coloured formazan) on exposure to light by superoxide radicals. It competes with enzyme SOD for superoxide anions. In presence of SOD in reaction mixture, NBT will produce less amount of coloured complex than control (therefore less OD than reference or control without enzyme).

Solution

1. 0.1M Phosphate buffer containing 0.5 mM E.D.T.A
2. 100 mM Phosphate buffer
3. 200 mM methionine
4. 2.25 mM NBT
5. 1.5 M Sodium carbonate
6. 2µM riboflavin

NOTE: Mix 2, 3, 4, 5 solutions in a beaker to form reaction mixture

Procedure

1. Leaf sample (0.1 g) was homogenized with pre-chilled 0.1M Phosphate buffer containing 0.5 mM E.D.T.A and vortex for 2-3 min
2. The homogenate was centrifuged at 15000 rpm @ 15 min at 4^{o}C
3. Pipetted out 200 µl supernatant (enzyme extract) in a test tube (duplicates)
4. Added 2ml reaction mixture to enzyme extract and make final volume to 3 ml by adding 0.8ml of ADW
5. The reaction was started by adding 400 µl of riboflavin to each test and placing the test tubes under two 18 W fluorescent lamps for 15 min
6. After 15 min reaction was terminated by switching off light, and tubes were kept in dark
7. The observations were recorded at 560 nm on a UV-vis spectrophotometer

Control: Complete reaction mixture without enzyme extract + light act as control

Blank: complete reaction mixture without enzyme extract – light act as blank.

Calculation:

Percent inhibition of NBT reduction by SOD is equal to (reference control OD-treatment OD/ reference control) x 100

The 50% inhibition is equal to 1 unit of enzyme.

Final formula for SOD units /g fresh weight of sample will be

$$\text{SOD Units /g FW} = \frac{C-T}{T} \times 100 \times \frac{1}{50} \times \frac{\text{System volume}}{\text{Enzyme extract}} \times DF \times \frac{1}{0.1}$$

Where C = reference control OD

T = treatment OD

System volume = 3 ml + 0.4 ml = 3400 µl

Enzyme extract volume = 200 µl

DF = dilution factor = 3000/200 = 15

0.1 = starting material weight 0.1 g

3.2.3.1.2 Measurement of lipid peroxidation (LPO)

Lipid peroxidation is oxidative degradation of lipid-fatty acids by reactive oxygen species and hence it is considered as one of the measures of oxidative stress in the cells. The method for lipid peroxidation estimation described by Ohkawa *et al*., 1979 is given as follows.

Principle

Oxidative degradation of lipid-fatty acids by reactive oxygen species increases the concentration of lipid hydroperoxides and aldehydes in the cells. These lipid hydroperoxides and aldehydes reacts with 2-thiobarbituric acid (TBA) hence called as Thiobarbituric acid reactive substances (TBARS). The TBARS content is measured in terms of malondialdehyde (MDA) which results from decomposition of the unstable peroxides of polyunsaturated fatty acids. MDA reacts with (TBA) resulting in the formation of a red colored complex with absorbance maxima at 532 nm (www.isca.in).

Solutions

- Trichloroacetic acid (TCA) (20 %)
- Thiobarbituric acid reagent: 1% TBA in 20% TCA

Procedure

1. To 0.1 gram of powder leaf sample adds 4.0 ml of 20% TCA containing 1% TBA in a test tube prepared in duplicates/replicates.
2. Heat the mixture for 30 min at 95° C in a water bath and immediately cool it in an ice bath to stop the reaction.
3. Centrifuge the cool samples at 10,000 g for 15 min.
4. Record the absorbance of the clear supernatant at 532 nm

Blank: A sample blank containing the 20% TCA containing 1% TBA without enzyme extracts.

Calculations : The MDA content can be calculated either from a standard curve of MDA (0- 20µM range) or based on the extinction coefficient of MDA 1.56 x $10^5 M^{-1}$ cm-1 or 156000 $M^{-1}cm^{-1}$. For MDA standard, replace sample with the dilutions of MDA. The MDA content is represented as n mols per g dry or fresh weight.

Formula:

As per Beer's Lambert Law

$A = \varepsilon l c$ i.e. $c = A/ \varepsilon l$

Where A = Absorbance of solution at a particular wavelength;

ε = Molar Absorptivity or molar extinction coefficient of MDA = 156000 $M^{-1}cm^{-1}$;

l = Length of Solution Cell = here 1 cm;

c = Concentration of Solution (mmol/L).

To covert c to nmole/L/g; multiply by 10^6 and divide by 0.1 (since starting material is 0.1 g leaf powder). Therefore, formula becomes

$$C = \frac{A \times 10^6}{\varepsilon \times l \times 0.1} \text{ nmole/L/g}$$

Same calculation can also be done using web browser

https://www.instanano.com/2017/01/Concentration-Calculation-UV-Vis-Absorbance.html

3.2.3.1.3 Measurement of peroxidase (POx) activity (E.C.1.11.1.7)

Peroxidase (POx) activity was measured spectrophotometrically as described by Hammerschmidt *et al.*, 1982 with pyrogallol as hydrogen donor.

Principle

Peroxidase also referred as non-specific peroxidase catalyses the reduction of hydrogen peroxide with a concurrent oxidation of pyrogallol to a colored purpurgallin. The increase in absorbance is recorded at 420 nm.

H_2O_2 + Pyrogallol(donor) $\xrightarrow{\text{peroxidase}}$ $2H_2O$ + purpurgallin(oxidized donor)

Solutions

0.05M Pyrogallol

0.1 M Phosphate buffer (pH7)

1% H_2O_2

Procedure

1. To 0.1 gram of powder leaf sample adds 5 ml ice cold 0.1 M Phosphate buffer (pH7) in a test tube prepared in duplicates/replicates.
2. Homogenate was vortex for 2-3 min for easy dispersion of leaf sample in buffer
3. The Homogenate was centrifuged @ 16000 rpm at 4^{o}C for 15 min and supernatant was used as crude enzyme source.
4. Take 50 µl of supernatant in a test tube (replicates) and add 1.5 ml of 0.05M Pyrogallol
5. Just before taking the observation at spectrophotometer add 0.5 ml of 1% H_2O_2
6. The observation at 420 nm was recorded at 30 s intervals for 3 min

Blank: A sample blank contains the 0.1 M Phosphate buffer, 0.05M Pyrogallol and 1 % H_2O_2without enzyme extracts.

Calculations

Peroxidase activity (U/L)

$$= \frac{\Delta Abs \text{ x } 100 \text{ x } \text{System volume x } 1}{\Delta t \text{ x } € \text{ x L Enzyme extract X } 0.1}$$

Where

ΔAbs = change in absorbance = Absorbance at 3 min-Absorbance at 0 min

Δt = change in time (in min, here 3 min)

L = path length or cuvette diameter (=1 cm)

€ = molar extinction coefficient of substrate in units of $M^{-1}cm^{-1}$ (here for pyrogallol its 12 $M^{-1}cm^{-1}$

Total assay volume (= 1.5 ml + 50 ul + 0.5 ml = 2050 ul)

Enzyme extract volume (= 50 ul)

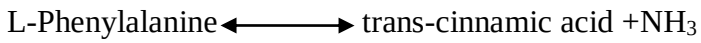
3.2.3.2 Determination of Phenylpropanoid activities.

3.2.3.2.1 Estimation of Phenylalanine ammonia-lyases (PAL) (E.C.4.1.3.5)

PAL activity was measured in terms of amounts of t-cinnamic acid (t-CA) formed according to the method of Brueske., 1980.

Principle

Phenylalanine ammonia lyase catalyzes the conversion of phenylalanine to trans-cinnamic acid and ammonia, as a step in the phenylpropanoid pathway of plants and is therefore involved in the biosynthesis of the polyphenol compounds such as flavonoids, phenolpropanoids and lignin in plants.



Solutions

0.1M Phosphate buffer containing 1.4 mM mercaptoethanol

0.1 mM L-Phenylalnine (pH8.7)

1M TCA

Procedure

1. One gram of leaf sample was homogenized in 2ml of ice cold 0.1 M Phosphate buffer having 1.4mM mercaptoethanol and votex.
2. Centrifuge at 16000 rpm at 4^{o}C for 15min
3. Pipet out 200 µl of supernatant (enzyme extract) into test tube (replicates)
4. Add 500 µl of 0.1 M phosphate buffer and 1.3 ml of ADW and mix it.
5. To initiate reaction, add 500 µl of 0.1mM L-Phenylalanine in each test tube and incubate at 32^{o}C for 30min
6. The reaction was stopped by adding 500 µl of 1M TCA
7. Record the observation at 290 nm

Blank: A sample blank containing the 0.1 M Phosphate buffer, 0.1 mM L-Phenylalnine and 1 M TCA without enzyme extracts.

Calculations

Trans-cinnamic acid content can be calculated either from a standard curve of trans-cinnamic acid or based on the extinction coefficient of trans-cinnamic acid. The trans-cinnamic acid content is represented as mmole/L/g fresh weight.

Formula:

As per Beer's Lambert law

$A = \varepsilon l c$ i.e. $c = A/ \varepsilon l$

where = Absorbance of solution at a particular wavelength

ε = Molar Absorptive or molar extinction coefficient of Trans cinnamic acid = 9630 $M^{-1}cm^{-1}$;

l = Length of Solution Cell = here 1 cm;

c = Concentration of Solution (mmol/L).

To covert c to mmole/L/g; divide by 0.1 (since starting material is 0.1 g leaf powder). Therefore, formula becomes

$$C = \frac{A}{\varepsilon \times l \times 0.1} \text{ mmole/L/g}$$

Same calculation can also be done using web browser

https://www.instanano.com/2017/01/Concentration-Calculation-UV-Vis-Absorbance.html

3.2.3.2.2 Polyphenol oxidase (PPO) (E.C.1.14.18.1)

PPO activity was evaluated as described by Gauillard *et al.*, 1973 with catechol as substrate for PPO.

Principle

Enzymatic oxidation of catechol by PPO/O_2 transformed the substrate into yellow product with a maximum absorbance at 495 nm. These enzymes are released by broken cells and they catalyse the reaction between colorless molecules called polyphenols and molecular oxygen this reaction creates colored compounds and these new compounds can spontaneously cross react with one another to form black-brown complexes called melanin's.

Catechol + oxygen $\xrightarrow{\text{polyphenioxidase}}$ Benzoquinone + water $\longrightarrow$ melanin

Solutions

0.1M Phosphate buffer containing 0.01M catechol (reaction mixture) (pH6.5)

Procedure

1. 0.1 gram of leaf sample was homogenized in 5ml of 0.1M Phosphate buffer
2. Homogenate was centrifuged at 16000 rpm for 30 min @ 4ºC
3. The reaction mixture contained 0.01 M catechol (0.4 ml) in 0.1 M sodium phosphate buffer (3.0 ml; pH 6.5).
4. At the time of taking observation 400 µl of enzyme, extract was added to reaction mixture and reading was taken.
5. The observation at 495 nm was recorded at 30sec intervals up to 3 min

Blank: A sample blank containing the 0.1M Phosphate buffer containing 0.01M catechol (reaction mixture) without enzyme extracts.

Calculations :

Polyphenol oxidase activity (U/L) = change in OD per minute per gram fresh weight

$$= \frac{\Delta Abs \times 100 \times \text{System volume} \times 1}{\Delta t \times € \times L \text{ Enzyme extract} \times 0.1}$$

Where

ΔAbs = change in absorbance = Absorbance at 3 min-Absorbance at 0 min

Δt = change in time (in min, here 3 min)

L = path length or cuvette diameter (=1 cm)

€ = molar extinction coefficient of substrate in units of $M^{-1}cm^{-1}$ (here for catechol its 3450 $M^{-1}cm^{-1}$

Total assay volume (0.4 ml + 3.0 ml + 400 µl = 3800 ul)

Enzyme extract volume (= 400 ul)

3.3.2.3 Total phenolic content (TPC)

TPC was determined as Zheng and Shetty., 2000.

Solutions

95 % ethanol

1N Folin-ciocalteau reagent

5% Na_2Co_3

Procedure

1. 0.1 gram of leaf sample was placed in 5 ml of 95% ethanol and kept at 0ºC for 48 hrs for maximum extraction
2. Each sample was then homogenized and centrifuge at 13000 rpm for 10 min
3. Pipet out 1 ml of supernatant and mix it with 1 ml of 95 % ethanol and 5 ml ADW
4. To this added 0.5 ml of 1 N Folin-ciocalteau reagent was added
5. After 5 min added 1 ml of 5% Na_2Co_3 and reaction mixture was allowed to stand for 60 min and the absorbance at 725 nm was recorded

Calibration curve was prepared for using various concentration of gallic acid (GA) in 95 % ethanol. The absorbance value was converted to µg or mM gallic acid equivalent (GAE) g/FW.

Blank: A sample blank contained95 % ethanol, 1N Folin-ciocalteau reagent and 5% Na_2Co_3 without enzyme extracts.

CHAPTER – IV
RESULTS AND DISCUSSION

Fungicides are known to produce fungicidal effects on fungal pathogens, but some fungicide like strobilurins have been reported to incite physiological changes in crops, modifications in the balance of phytohormones, like increase in tolerance against abiotic stress, delayed senescence of photosynthetic leaf area, increased Carbon dioxide uptake and darker green appearance of leaves. Strobilurins (Qo inhibitors) have been repeatedly shown to result in increased kernel weights, grain yields, and protein contents which are correlated with a delay in the senescence of flag leaves. One of the most positive side effects recorded was "Green effects" triggered by strobilurins. Kresoxim-methyl a derivative of fungal secondary metabolite strobilurin A inhibited mitochondrial respiration (Sauter *et al.,* 1995; Anke, 1995) and also was found to induce non-fungicidal physiological changes in wheat (Grossmann and Retzlaff, 1997) like darker green appearance of the leaves, delayed leaf senescence, increased stress tolerance and favored plant biomass and corn production. Kresoxim-methyl had also been reported to improve photosynthetic efficiency, influences the hormonal status of the plant through a bioregulatory auxin-like activity. Present investigations were therefore undertaken to investigate the effects of Kresoxim-methyl on three crops wheat, rice and chickpea. Experiments were conducted under field and also under disease-free conditions in the rainout shelters. Experiment was conducted to see the effect of four increasing doses of Kresoxim-methyl on germination, and radicle morphology of wheat, rice and chickpea. Growth promoting effects observed on germination and radicle morphology of crops also prompted us to undertake with an argument that because Kresoxim-methyl displays a similar mode of action to that of plant growth-promoting bacteria. The dual effect of fluorescent

Pseudomonas applied through seed treatment and Kresoxim-methyl as spray during the subsequent growth stages of crop on plant growth stimulation was investigated. Two fluorescent *Pseudomonas* isolates 9704 and 9829 were used. The test crops were sprayed with four increasing doses of Kresoxim-methyl and observations were recorded on different plant morphological parameters.

4.1 Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on germination on wheat, rice and chickpea

The treatment of seed with fungicide is required to achieve the greatest net benefit like enhanced emergence in seedling, plant vigor, and plant and the height of the plant, root biomass through protection from seed-borne and soil-borne pathogens (Anderson and Buzzell 1982; Guy *et al.,* 1989; Dorrance and McClure 2001; da Silva *et al.,* 2017). Various inherent benefits of fungicide seed treatment have been reported (Khangura and Barbetti 2004, Khanzada *et al.,* 2002, Sundin *et al.,* 1999, Greenhalgh *et al.,* 1994, Greenhalgh and Clarke, 1985).

Wheat, chickpea and rice seeds were treated with four doses of Kresoxim-methyl (44.3% SC): 310.17 ppm(14µl/20ml of SDW), 354.48 ppm (16µl/20ml of SDW), 398.29 ppm(18µl/20ml of SDW) and 443 ppm (20µl/20ml of SDW) and subjected to seed germination test.

The results shown inPlates 1, 2 & 3on frequency of germination in chickpea, wheat and paddy indicated that it was higher than control on 1st day, 3rd day and 5th day of incubation in case of treated seeds. Differences in radicle lengths were larger than control. Apparent differences in the radicle length were observed on third day of observation. It was also observed that radicle of germinating seeds of wheat was covered with very fine hairy roots whereas in control the radicle of germinating seeds was not covered with fine root hairs. Similarly, the germination derived that paddy seeds treated with Kresoxim-methyl have induced higher frequency of root hairs and lateral roots as compared to untreated control. The frequency of root hairs and lateral roots increased in radicle of germinated seedlings with the increase in doses of Kresoxim-methyl (Plate 1, 2 & 3).

Table 4.1: - Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on germination on wheat, rice and chickpea

S. No.	Doses of Kresoxim-methyl*	Germination out of 30 seeds				
		0 day	1 day	3 day	5 day	7 day
	Chickpea					
1	**F20**	0	8	27	27	29
2	**F18**	0	9	23	30	30
3	**F16**	0	11	14	25	28
4	**F14**	0	9	19	23	28
5	**Control**	0	4	13	19	25
	Wheat					
1	**F20**	0	7	18	30	30
2	**F18**	0	5	18	28	30
3	**F16**	0	7	17	23	28
4	**F14**	0	7	14	27	27
5	**Control**	0	3	11	17	27
	Rice					
1	**F20**	0	6	17	30	30
2	**F18**	0	5	13	29	30
3	**F16**	0	4	14	24	28
4	**F14**	0	7	15	26	27
5	**Control**	0	4	13	22	29

***** **F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW); **F18**=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

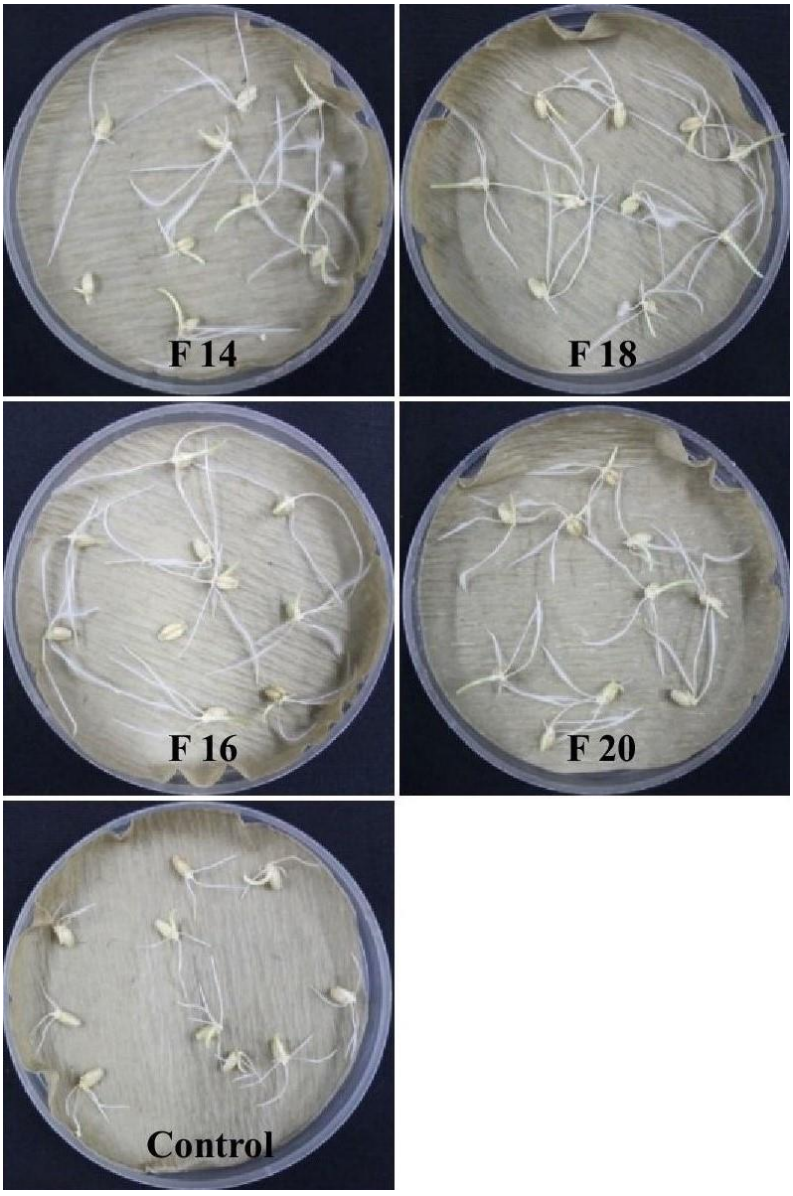


Plate 4.1(a): Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on germination on wheat

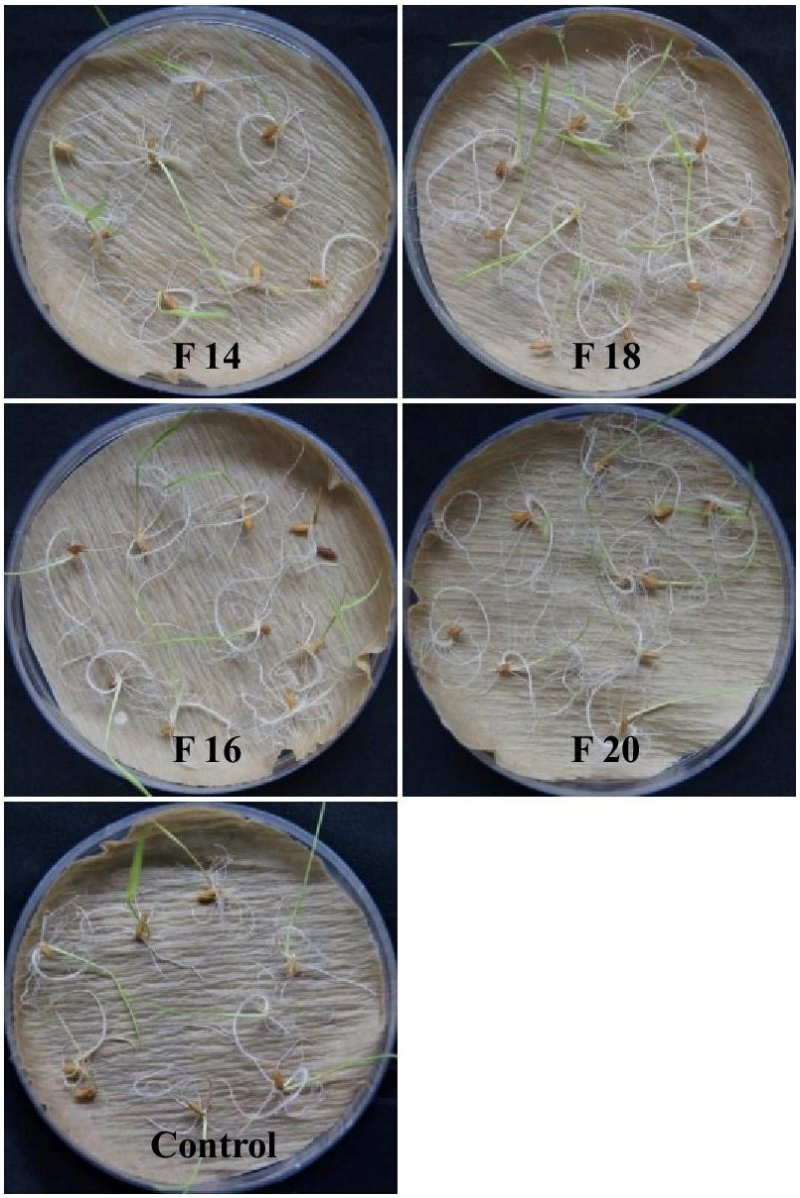


Plate 4.1(b): Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on germination on rice

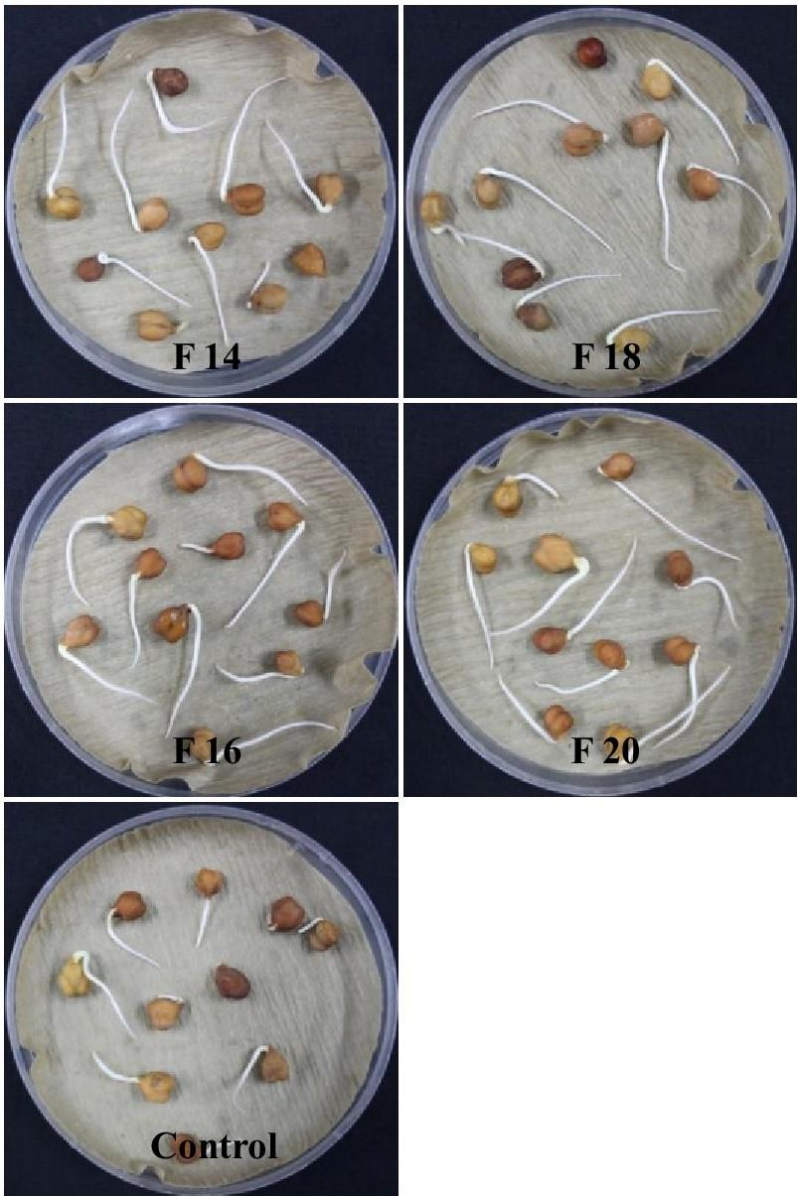


Plate 4.1(c): Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on germination on chickpea

4.2 Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl different crops

Customarily, for controlling and preventing diseases, fungicides are used. But, a group of fungicides termed as strobilurins have brought a new advantage of , physiological impacts through alterations in metabolism and growth, resulting in the increased crop yields. Kresoxim-methyl has been discovered to influence physiological modifications in several crops along with a positive influence on yield (Gold *et al.,* 1995). Following the application of Kresoxim-methyl, the level of 1-aminocyclopropane1-carboxylic acid (ACC), an ethylene precursor, and ACC synthase activity showed a concentration-dependent decrease and a subsequent reduction in ethylene production. Lower ethylene concentrations have been shown to slow degradation of cytokinins (Bollmark, & Eliasson, 1990), which have been shown to delayed senescence (Badenoch-Jones *et al.,* 1996), and increased chlorophyll production (Kuroda *et al.,* 1996, Sundqvist *et al.,* 1980). The increase in growth, yield, and photosynthesis rate in plants treated with Kresoxim-methyl may be due to to reduce chlorophyll degradation, greater assimilate concentrations, and delayed senescence. Although plant growth-promoting bacteria used a number of different mechanisms to promote the growth of plants (Glick 2012), arguably, the bacterial trait that is key in facilitating plant growth is the possession of the enzyme 1-aminocyclopropane1-carboxylate (ACC) deaminase. This enzyme is responsible for the cleavage of the plant ethylene precursor, ACC, into ammonia and - ketobutyrate (Honma and Shimomura 1978). The ACC deaminase-producing organisms decrease plant ethylene levels by decreasing ACC levels in plants (Glick *et al.,* 1998, 2007), which may lead to plant growth inhibition or even death when present at high concentrations.

The present investigations were therefore undertaken with an hypothesis that Kresoxim-methyl displays a similar mode of action with that of plant growth-promoting bacteria. The answer of question whether the combination of application (Fluorescent *Pseudomonas* and Kresoxim-methyl) have a significant positive effect on plant metabolism resulting in growth and yield in different crops was explored. In

addition to itinvestigations were also madeon any dual effect of fluorescent *Pseudomonas* applied through seed treatment and Kresoxim-methyl as spray during the subsequent growth stages of crop on plant growth stimulation. Two isolates of fluorescent *Pseudomonas* namely, 9704 and 9829 were used. Wheat, chickpea and rice plants derived from seed treatment with fluorescent *Pseudomonas* isolates were treated with four doses of Kresoxim-methyl (44.3% SC): 310.17 ppm(14µl/20ml of SDW), 354.48 ppm (16µl/20ml of SDW), 398.29 ppm(18µl/20ml of SDW) and 443 ppm (20µl/20ml of SDW). Observations were recorded on different plant morphological parameters.

4.2.1 Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on wheat

The potential biostimulant activity of wheat plant sprayed Kresoxim-methyl was investigated. To this end, a bioassay (Audus test) carried out to determine the effect of Kresoxim-methyl, morphological variations in wheat plants at different fungicidal doses were measured in pot conditions.

The sprays of Kresoxim-methylon wheat plant significantly modified morphological traits and significant increase in root length, root volume, shoot length, number of tillers and grain yields were recorded. Such increase in different morphological characters and grain yield was in proportion to the doses of Kresoxim-methyl in different treatment (Tables 4.2 (a-e).

1. **Effects of Kresoxim-methyl on root length of wheat**: -

Plants sprayed with443 ppm concentration of of Kresoxim-methyl expressed 33.33% increases in root length over control. Similarly, plants derived from seed bacterization with 9829 and sprayed with 398.29 ppm dose of Kresoxim-methylgot 30.30% increase in root length and plants derived from seed bacterization with 9704 and sprayed with 398.29 ppm dose of Kresoxim-methyl had 31.81% increased root length over untreated control.

2. Effects of Kresoxim-methyl on root volume of wheat:-

The plants sprayed with 443ppm dose of Kresoxim-methyl expressed 36.38% increase in root volume over control. Similarly, plants raised using seed bacterization with 9829 and later sprayed with 443 ppm dose of Kresoxim-methyl gave30.76% increase in root volume. Likewise, the plants derived from seed bacterization with 9704 followed by spray with 443 ppm and 398.29 ppm doses of Kresoxim-methyl expressed 57.98 and 51.99% increase in root volume over control. Significantly enhanced root volume expressed in treatments where the seed were bacterized with 9704 followed by fungicide spray, as compared to the plants which were only sprayed with fungicide.

3. Effects of Kresoxim-methyl on shoot length of wheat: -

Plants sprayed with 443 ppm dose of Kresoxim-methyl expressed 10.69 % increase in shoot length over control. Similarly, plants derived from seed bacterization with 9829 and 9704 and sprayed with 443 ppm dose of Kresoxim-methyl expressed 10.69 and 14.57% increase in shoot length over control. Significant enhanced shoot length was expressed in treatments where the seed were bacterized with 9704 followed by fungicide spray, as compared to the plant which was only sprayed with fungicide and control.

4. Effects of Kresoxim-methyl on number of tillers of wheat:-

Plants sprayed with 443 ppm and 398.29 ppm dose of Kresoxim-methyl expressed 58.33 and 41.67 % increase in number of tillers induced over control. Similarly, plants derived from seed bacterization with isolate 9829 and sprayed with four increasing doses of Kresoxim-methyl expressed less increase in induced number of tillers as compared to plants which were only sprayed with fungicide and plant which were derived from seed bacterization with isolate 9704 and sprayed with four increasing doses of Kresoxim-methyl. Significant enhanced number of tillers was expressed in treatments where the seed were bacterized with 9704 followed by fungicide spray, as compared to the plant which was only sprayed with fungicide and control. Increase in number of tillers induced (66.67 and 50.00% respectively) where

wheat seeds were bacterized with isolate 9704 followed by fungicide spray with 443 ppm and 398.29 ppm dose of Kresoxim-methyl.

5. Effects of Kresoxim-methyl on yield of wheat:-

Plants sprayed with 443 ppm and 398.29 ppm dose of Kresoxim-methyl expressed 58.04 and 61.71 % increase in yield over control. Dose 398.29 ppm of Kresoxim-methyl expressed more yield in pot trials as compared to higher dose of Kresoxim-methyl. Similarly, plants derived from seed bacterization with isolates 9829 and 9704 and sprayed with 443 ppm and 398.29 ppm dose of Kresoxim-methyl expressed larger effects on yield increase (57.23, 55.87% and 65.35 and 54.98% respectively) as compared to control. Combination of seed bacterization followed with spray application of Kresoxim-methyl did not resulted in significant differences as compared to the yield derived from plants prayed only with Kresoxim-methyl.

Table 4.2(a): - Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting root length in wheat

S. No.	Treatment*	Root Length (cm)	S. No.	Treatment	Root Length (cm)	S. No.	Treatment	Root Length (cm)
1	**F-20**	29.333±1.453	**5**	**9829- F-20**	27±1	**9**	**9704- F-20**	24.333±4.807
2	**F-18**	28±3.464	**6**	**9829- F-18**	28.667±2.404	**10**	**9704- F-18**	29±0.577
3	**F-16**	27±2.517	**7**	**9829- F-16**	26.333±0.882	**11**	**9704- F-16**	27±1.155
4	**F-14**	24.667±3.844	**8**	**9829- F-14**	25.333±0.882	**12**	**9704- F-14**	25±2.517
						13	**Control**	22±2.887
	C.D	**N/A**	**SE(m)**	2.527	**SE(d)**	3.573	**C.V.**	16.555
	F value	0.703						
				Per cent increase over control				
1	**F-20**	33.33333	**5**	**9829- F-20**	22.72727	**9**	**9704- F-20**	10.60606
2	**F-18**	27.27273	**6**	**9829- F-18**	30.30303	**10**	**9704- F-18**	31.81818
3	**F-16**	22.72727	**7**	**9829- F-16**	19.69697	**11**	**9704- F-16**	22.72727
4	**F-14**	12.12121	**8**	**9829- F-14**	15.15152	**12**	**9704- F-14**	13.63636

***F14** = 310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW); **F18**=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Table 4.2(b): - Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting root volume in wheat

S. No.	Treatment*	Root vol. ($cm^{3)}$	S. No.	Treatment	Root vol. ($cm^{3)}$	S. No.	Treatment	Root vol. ($cm^{3)}$
1	**F-20**	37.687^{ABC}±2.265	**5**	**9829- F20**	36.133^{ABC}±3.977	**9**	**9704- F20**	43.657^{A}±5.768
2	**F-18**	37.58^{ABC}±4.42	**6**	**9829- F18**	33.157^{ABCD}±5.821	**10**	**9704- F18**	42^{AB}±4.42
3	**F-16**	30.947^{BCD}±4.818	**7**	**9829- F16**	26.527^{CD}±3.317	**11**	**9704- F16**	33.157^{ABCD}±4.977
4	**F-14**	28.74^{CD}±2.21	**8**	**9829- F14**	26.527^{CD}±3.317	**12**	**9704- F14**	23.763^{D}±1.464
						13	**Control**	27.633^{CD}±2.925
	C.D	11.825	**SE(m)**	4.045	**SE(d)**	5.721	**C.V.**	21.307
	F value	2.381						
				Per cent increase over control				
1	**F-20**	36.38283	**5**	**9829- F-20**	30.76153	**9**	**9704- F-20**	57.98743
2	**F-18**	35.99682	**6**	**9829- F-18**	19.98938	**10**	**9704- F-18**	51.99218
3	**F-16**	11.9917	**7**	**9829- F-16**	(-) 4.00367	**11**	**9704- F-16**	19.98938
4	**F-14**	4.00608	**8**	**9829- F-14**	(-) 4.00367	**12**	**9704- F-14**	(-) 14.0038

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW); **F18**=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Table 4.2 (c): - Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting shoot length in wheat

S. No.	Treatments*	Shoot Length (cm)	S. No.	Treatments	Shoot Length (cm)	S. No.	Treatments	Shoot Length (cm)
1	**F-20**	$38A^{B}\pm0.577$	**5**	**9829-F-20**	$38^{AB}\pm0.577$	**9**	**9704- F-20**	$39.333^{A}\pm0.882$
2	**F-18**	$37.333^{ABC}\pm0.882$	**6**	**9829-F-18**	$37^{BC}\pm1$	**10**	**9704- F-18**	$37.667^{ABC}\pm0.882$
3	**F-16**	$36.667^{ABC}\pm0.882$	**7**	**9829-F-16**	$37^{BC}\pm0.577$	**11**	**9704- F-16**	$37^{BC}\pm1$
4	**F-14**	$35.667^{CD}\pm0.882$	**8**	**9829-F-14**	$36.333^{BCD}\pm0.333$	**12**	**9704- F-14**	$36.667^{BC}\pm0.333$
						13	**Control**	$34.333^{D}\pm0.882$
	C.D	2.277	**SE(m)**	0.779	**SE(d)**	1.102	**C.V.**	3.647
	F value	2.411						
Per cent increase over control								
1	**F-20**	10.69	**5**	**9829- F-20**	10.69	**9**	**9704- F-20**	14.57
2	**F-18**	8.75	**6**	**9829- F-18**	7.78	**10**	**9704- F-18**	9.72
3	**F-16**	6.81	**7**	**9829- F-16**	7.78	**11**	**9704- F-16**	7.78
4	**F-14**	3.89	**8**	**9829- F-14**	5.84	**12**	**9704- F-14**	6.81

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW); **F18**=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Table 4.2(d): - Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting number of tillers in wheat

S. No.	Treatments*	Tillers No.	S. No.	Treatments	Tillers No.	S. No.	Treatments	Tillers No.
1	**F-20**	$6.333^{AB}\pm0.333$	**5**	**9829- F-20**	$4.667^{CDEF}\pm0.667$	**9**	**9704- F-20**	$6.667^{A}\pm0.333$
2	**F-18**	$5.667^{ABCD}\pm0.667$	**6**	**9829- F-18**	$5.333^{ABCDE}\pm0.333$	**10**	**9704- F-18**	$6^{ABC}\pm0$
3	**F-16**	$4.333^{DEF}\pm0.333$	**7**	**9829- F-16**	$4.667^{CDEF}\pm0.333$	**11**	**9704- F-16**	$5B^{CDEF}\pm0.577$
4	**F-14**	$4^{EF}\pm0.577$	**8**	**9829- F-14**	$4.333^{DEF}\pm0.882$	**12**	**9704- F-14**	$4^{EF}\pm0.577$
						13	**Control**	$3.667^{F}\pm0.333$
	C.D	1.48	**SE(m)**	0.506	**SE(d)**	0.716	**C.V.**	17.632
	F value	3.572						
				Per cent increase over control				
1	**F-20**	58.33	**5**	**9829- F-20**	16.67	**9**	**9704- F-20**	66.67
2	**F-18**	41.67	**6**	**9829- F-18**	33.33	**10**	**9704- F-18**	50.00
3	**F-16**	8.33	**7**	**9829- F-16**	16.67	**11**	**9704- F-16**	25.00
4	**F-14**	0.00	**8**	**9829- F-14**	8.33	**12**	**9704- F-14**	0.00

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW); **F18**=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Table 4.2(e): - Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting yield of wheat

S. No.	Treatments*	Yield (g)	S. No.	Treatments	Yield (g)	S. No.	Treatments	Yield (g)
1	**F-20**	17.227^{ABC}±0.398	**5**	**9829- F-20**	17.137^{ABC}±0.092	**9**	**9704- F-20**	18.023^{A}±0.694
2	**F-18**	17.627^{AB}±1.063	**6**	**9829- F-18**	16.99^{ABCD}±0.436	**10**	**9704- F-18**	16.893^{ABCD}±0.381
3	**F-16**	16.12^{BCDE}±0.496	**7**	**9829- F-16**	15.43^{DEF}±0.646	**11**	**9704- F-16**	15.933^{CDE}±0.347
4	**F-14**	15.06^{EFG}±0.396	**8**	**9829- F-14**	14.287^{FG}±0.346	**12**	**9704- F-14**	13.787^{G}±0.828
						13	**Control**	10.897^{H}±0.396
	C.D	1.625	**SE(m)**	0.556	**SE(d)**	0.786	**C.V.**	6.094
	F value	12.377						
				Per cent increase over control				
1	**F-20**	58.04281	**5**	**9829- F-20**	57.21713	**9**	**9704- F-20**	65.35168
2	**F-18**	61.71254	**6**	**9829- F-18**	55.87156	**10**	**9704- F-18**	54.98471
3	**F-16**	47.88991	**7**	**9829- F-16**	41.55963	**11**	**9704- F-16**	46.17737
4	**F-14**	38.16514	**8**	**9829- F-14**	31.07034	**12**	**9704- F-14**	26.48318

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW); **F18**=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

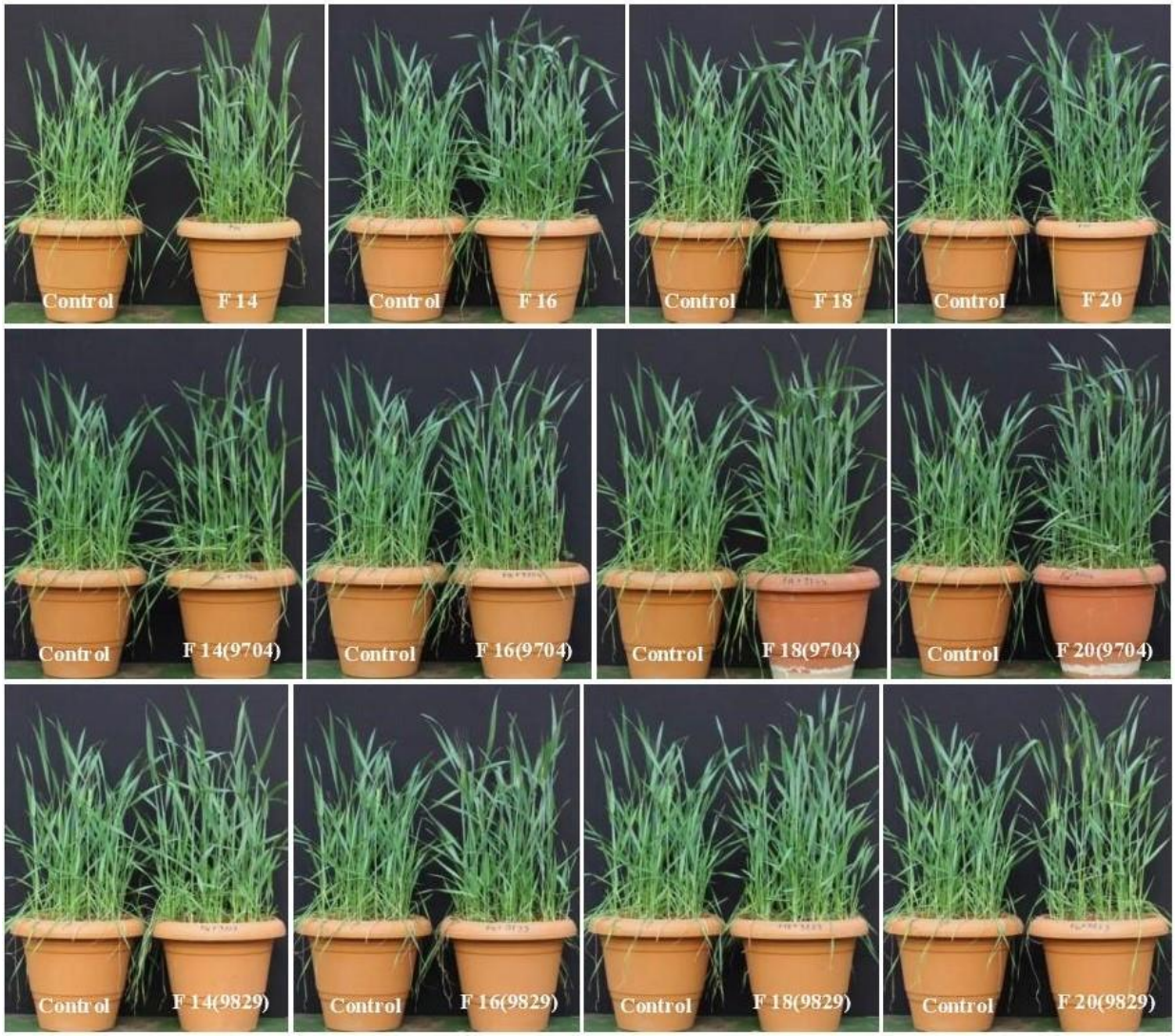


Plate 4.2 (a): Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on wheat

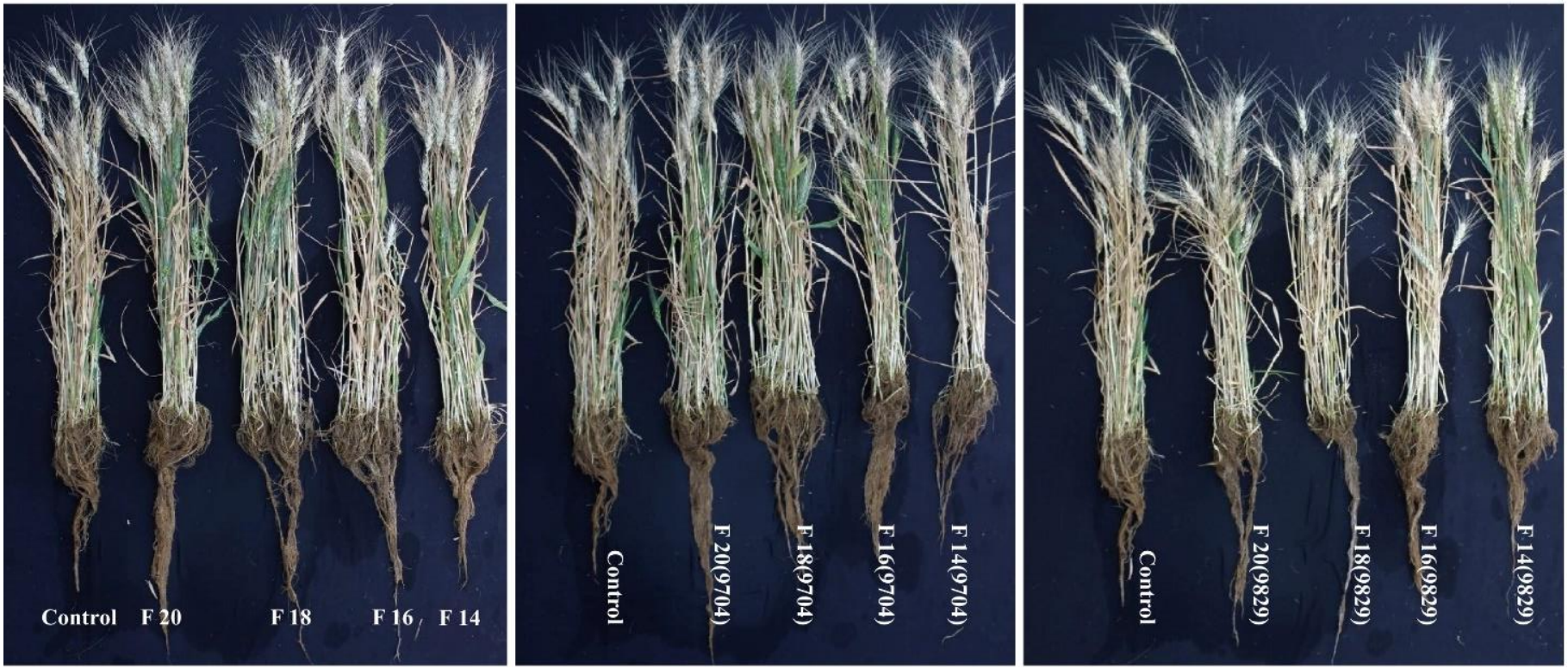


Plate 4.2 (b): Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on wheat

Plate 4.2 (c): Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on root of wheat

4.2.2 Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on rice

The effects of Kresoxim-methyl in rice was studied onmodifiecations of morphological traits. Although the improvement in root length, root volume, shoot length detected in this trial but it was not significant. The increase in root length, root volume, shoot length,number of tillers and yield was almost in proportion to the doses of Kresoxim-methyl in different treatment combinations (Table 4.3a-c).

1. Effects of Kresoxim-methyl on root length of rice:-

Plants sprayed with 443 ppm dose of Kresoxim-methyl increased root length by 53.87% over control. Similarly, plants derived from seed bacterization with isolates 9829 and 9704 and sprayed with 443 ppm and 398.29 ppm doses of Kresoxim-methyl had 55.80, 59.80% and 65.41, 42.33% increase in root length over control, respectively. Highest increase in root length among treatments was 65.41% in case of isolate 9704 + 443ppm Kresoxim-methyl spray.

2. **Effects of Kresoxim-methyl on root volume of rice:-**

Plants The root volume of plants sprayed with 443 ppm dose of Kresoxim-methyl increased by 58.46% over control. Likewise, the seed bacterization with 9829 and spray of 443 ppm of Kresoxim-methyl gave 34.08% increase in root volume. Plants derived from seed bacterization with 9704 and sprayed with 443ppm dose of Kresoxim-methyl expressed 60.90% increased root volume over control. Enhanced root volume was observed in treatments where the seeds were bacterized with isolate 9704 followed by fungicidal spray, as compared to the plants which were sprayed with fungicide only.

3. **Effects of Kresoxim-methyl on shoot length of rice:-**

Shoot length was not much influenced by spray application of Kresoxim-methyl. Plants sprayed with 443 ppm and 398.29 ppm dose of Kresoxim-methyl expressed 19.98 and 18.93% increase in shoot length over control. Similarly, plants derived from seed bacterization with isolates 9829 and 9704 and sprayed with 443 ppm dose of Kresoxim-methyl expressed 16.82 and 23.14% increase in shoot length over control. Enhanced shoot length was expressed in treatments where the seed were bacterized with isolate 9704 followed by fungicide spray, as compared

to the plant which was only sprayed with fungicide and control. Whereas, shoot length was expressed comparatively less in treatments where the seeds were bacterized with isolate 9829 followed by fungicide spray, as compared to the plant which were only sprayed with fungicide and control.

Table 4.3(a): - Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting root length of rice

S. No.	Treatments*	Root length (cm)	S. No.	Treatments	Root length (cm)	S. No.	Treatments	Root length (cm)
1	**F-20**	26.667±4.333	**5**	**9829- F-20**	27±1.155	**9**	**9704- F-20**	28.667±2.404
2	**F-18**	23.667±4.372	**6**	**9829- F-18**	27±2.309	**10**	**9704- F-18**	24.667±4.096
3	**F-16**	23±2.646	**7**	**9829- F-16**	22.667±4.096	**11**	**9704- F-16**	21.667±1.856
4	**F-14**	21.667±2.028	**8**	**9829- F-14**	21.333±4.055	**12**	**9704- F-14**	21±3.215
						13	**Control**	17.333±4.91
	C.D.	N/A	**SE(m)**	3.389	**SE(d)**	4.793	**C. V.**	24.912
	F Cal	0.865						
Per cent increase over control								
1	**F-20**	53.87575	**5**	**9829- F-20**	55.79919	**9**	**9704- F-20**	65.41643
2	**F-18**	36.56472	**6**	**9829- F-18**	55.79919	**10**	**9704- F-18**	42.33506
3	**F-16**	32.71783	**7**	**9829- F-16**	30.79438	**11**	**9704- F-16**	25.02404
4	**F-14**	25.02404	**8**	**9829- F-14**	23.1006	**12**	**9704- F-14**	21.17715

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW);
F18=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Table 4.3 (b): - Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting root volume of rice

S. No.	Treatments*	Root vol. (cm^3)	S. No.	Treatments	Root vol. (cm^3)	S. No.	Treatments	Root vol. (cm^3)
1	**F-20**	15.308±4.512	**5**	**9829- F-20**	12.953±4.246	**9**	**9704- F-20**	15.543±2.855
2	**F-18**	11.775±1.928	**6**	**9829- F-18**	12.011±1.778	**10**	**9704- F-18**	11.775±1.928
3	**F-16**	10.598±1.471	**7**	**9829- F-16**	10.598±2.547	**11**	**9704- F-16**	11.54±2.093
4	**F-14**	8.478±0.816	**8**	**9829- F-14**	8.478±0.816	**12**	**9704- F-14**	10.598±1.471
						13	**Control**	9.655±1.928
	C.D.	N/A	**SE(m)**	2.44	**SE(d)**	3.45	**C. V.**	36.79
	F Cal	0.805						
				Per cent increase over control				
1	**F-20**	58.46273	**5**	**9829- F-20**	34.08385	**9**	**9704- F-20**	60.90062
2	**F-18**	21.89441	**6**	**9829- F-18**	24.3323	**10**	**9704- F-18**	21.89441
3	**F-16**	9.704969	**7**	**9829- F-16**	9.704969	**11**	**9704- F-16**	19.45652
4	**F-14**	(-)12.236	**8**	**9829- F-14**	(-)12.236	**12**	**9704- F-14**	9.704969

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW);
F18=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Table 4.3 (c):-Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting shoot length of rice

S. No.	Treatments*	Shoot length (cm)	S. No.	Treatments	Shoot length (cm)	S. No.	Treatments	Shoot length (cm)
1	**F-20**	38±1.732	**5**	**9829- F-20**	37±4.041	**9**	**9704- F-20**	39±1.155
2	**F-18**	37.667±1.856	**6**	**9829- F-18**	37±4.041	**10**	**9704- F-18**	36.667±2.603
3	**F-16**	36.667±2.028	**7**	**9829- F-16**	35±2.887	**11**	**9704- F-16**	36.333±3.48
4	**F-14**	37±3.215	**8**	**9829- F-14**	34.667±2.186	**12**	**9704- F-14**	34.333±1.333
						13	**Control**	31.667±2.404
	C.D.	N/A	**SE(m)**	2.695	**SE(d)**	3.812	**C. V.**	12.885
	F Cal	0.498						
				Per cent increase over control				
1	**F-20**	19.98737	**5**	**9829- F-20**	16.82981	**9**	**9704- F-20**	23.14493
2	**F-18**	18.93485	**6**	**9829- F-18**	16.82981	**10**	**9704- F-18**	15.77729
3	**F-16**	15.77729	**7**	**9829- F-16**	10.51468	**11**	**9704- F-16**	14.72477
4	**F-14**	16.82981	**8**	**9829- F-14**	9.462162	**12**	**9704- F-14**	8.409641

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW); **F18**=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

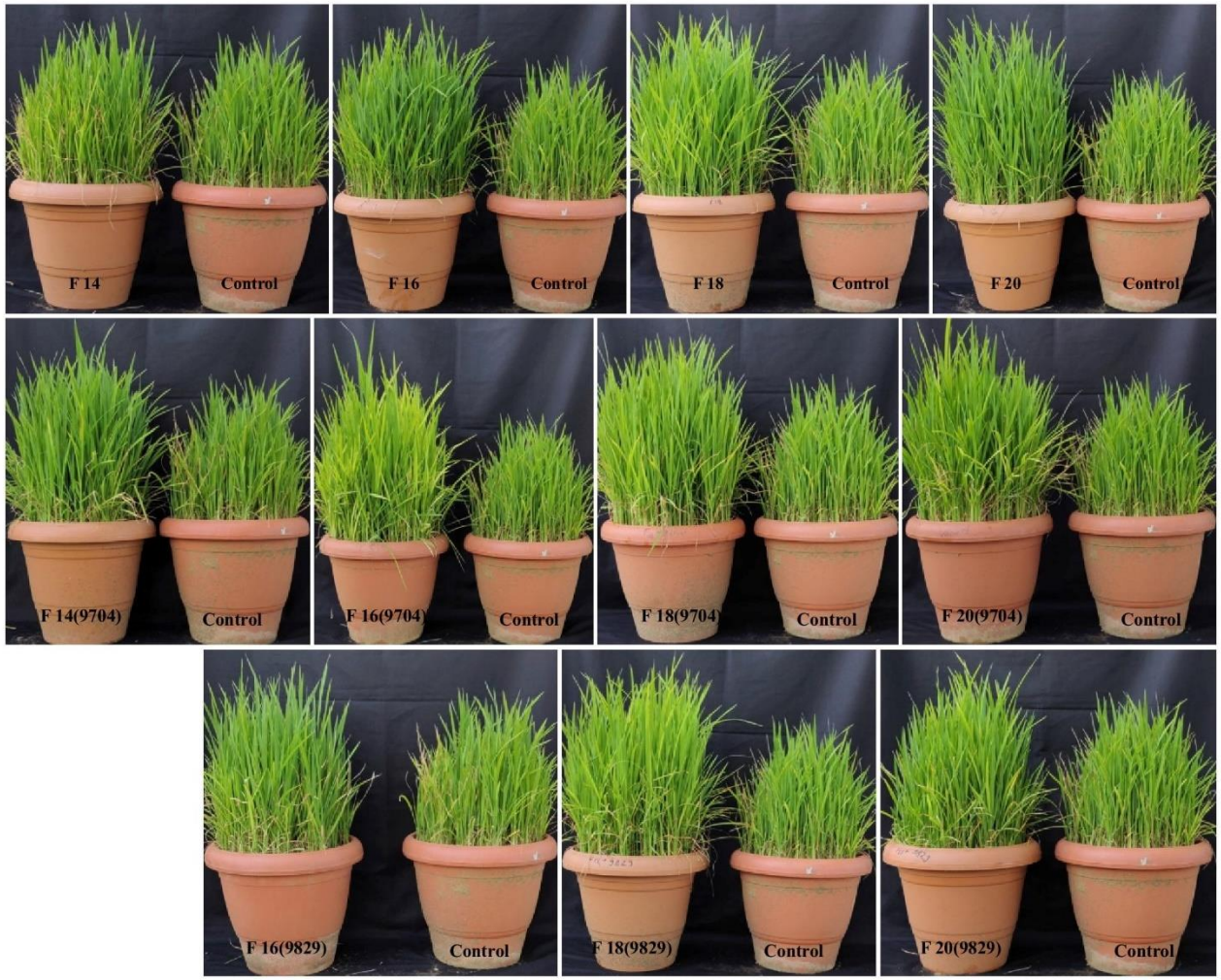


Plate 4.3(a): Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on rice

Plate 4.3(b): Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on rice

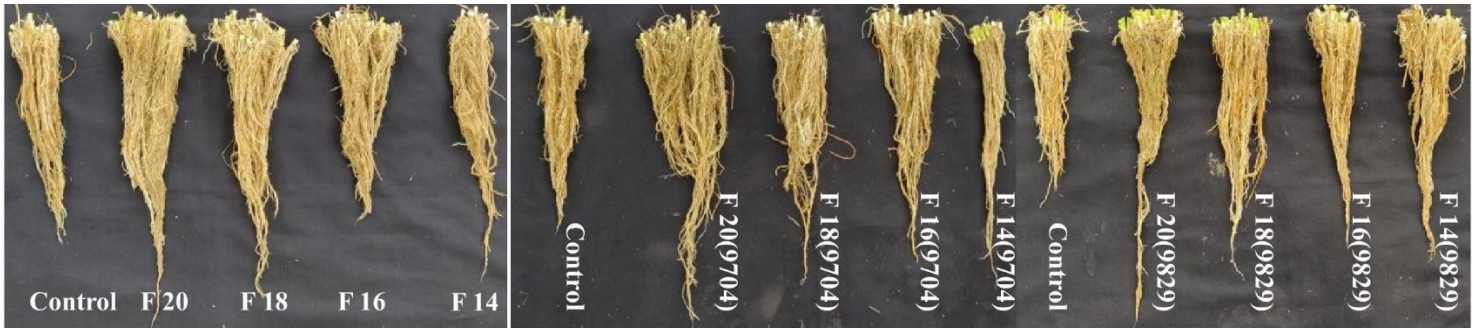


Plate 4.3(c): Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting root of rice

4.3 Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on rice WinRHIZO

We investigated the effects of Kresoxim-methyl in rice root morphology and found that foliar application significantly modified root morphological traits in disease-free plants. Significant differences in root length, root volume, surface area, number of root tips, number of forks detected in this trial as compared to control. It was observed that root length, root volume, surface area, number of root tips, number of forks increased almost in proportion to the dose of Kresoxim-methyl as compared to control(Table 4.4(a),(b), (c), (d), and (e)).

1. Effects of Kresoxim-methyl on root length of rice:-

Comparison of all treatment combination are detailed in table 4.4 (a) for the effect of Kresoxim-methyl on root length of rice. Plants sprayed with 443 ppm 398.29 ppm dose of Kresoxim-methyl expressed 71.39 and 63.17 % increase in root length over control. Similarly, plants derived from seed bacterization with isolates 9704 and 9829 and sprayed with 443 ppm and 398.29 ppm dose of Kresoxim-methyl expressed 105.57,125.95% and 107.44, 110.66% respectively increase in root length over control. Both the treatment combination expressed higher increase in root length over all treatment receiving only Kresoxim-methyl spray.

2. Effects of Kresoxim-methyl on root volume of rice: -

Comparison of all treatment combination are detailed in table 4.4 (b) for the effect of Kresoxim-methyl on root volume of rice. Plants sprayed with 443 ppm 398.29 ppm dose of Kresoxim-methyl expressed 58.37 and 73.17% increase in root volume over control. Dose of 398.29 ppm of Kresoxim-methyl induced larger effects on root volume as compared to 443 ppm dose. Similarly, plants derived from seed bacterization with isolate 9829 and sprayed with 443 ppm 398.29 dose of Kresoxim-methyl expressed 78.84 and 95.71% increase in root volume, but the dose of 398.29 ppm of Kresoxim-methyl induced larger effects on root volume as compared

to 443 ppm dose. Plants derived from seed bacterization with isolate 9704 and sprayed with 443 ppm 398.29 dose of Kresoxim-methyl expressed highest increase in root volume among all the treatment combinations. An increase of 153.39 and 125.31% in root volume was measured using WinRHIZO.

3. Effects of Kresoxim-methyl on root surface area of rice: -

Comparison of all treatment combination is detailed in table 4.4 (c) for the effect of Kresoxim-methyl on root surface area of rice. Plants sprayed with 443 ppm 398.29 ppm dose of Kresoxim-methyl expressed 64.68 and 68.04% increase in root surface area over control. Similarly, plants derived from seed bacterization with isolate 9829 and sprayed with 443 ppm 398.29 dose of Kresoxim-methyl expressed 92.55 and 103.02% increase in root surface area, but the dose of 398.29 ppm of Kresoxim-methyl induced larger effects on root surface area as compared to 443 ppm dose. Plants derived from seed bacterization with isolate 9704 and sprayed with 443 ppm 398.29 dose of Kresoxim-methyl expressed highest increase in root surface area among all the treatment combinations. An increase of 128.18 and 125.56% in root surface area was measured using WinRHIZO.

4. Effects of Kresoxim-methyl on number of root tips of rice:-

Comparison of all treatment combination is detailed in table 4.4 (d) for the effect of Kresoxim-methyl on number of root tips of rice. Plants sprayed with 443 ppm 398.29 ppm dose of Kresoxim-methyl expressed 93.14 and 64.49% increase in number of root tips over control. Similarly, plants derived from seed bacterization with isolate 9829 and sprayed with 443 ppm 398.29 dose of Kresoxim-methyl expressed 72.77 and 72.24% increase in number of root tips. Plants derived from seed bacterization with isolate 9704 and sprayed with 443 ppm 398.29 dose of Kresoxim-methyl expressed increase in number of root tips. An increase of 69.01 and 61.75% in number of root tips was measured using WinRHIZO. Highest frequency of number of root tips (93.14% increase) was measured in only fungicide sprayed plants

with 443 ppm dose of Kresoxim-methyl, which was highest amongst all the treatment combinations.

5. Effects of Kresoxim-methyl on number of forks in rice roots:-

Comparison of all treatment combination is detailed in table 4.4 (e) for the effect of Kresoxim-methyl on number of forks in rice roots. Plants sprayed with 443 ppm 398.29 ppm dose of Kresoxim-methyl expressed 119.71 and 110.53% increase in number of forks over control. Highest frequency of number of forks (119.71% increase) was measured in only fungicide sprayed plants with 443 ppm dose of Kresoxim-methyl, which was highest amongst all the treatment combinations. Plants derived from seed bacterization with isolate 9829 and sprayed with 443 ppm 398.29 dose of Kresoxim-methyl expressed 81.72 and 112.86% increase in number of forks. Plants derived from seed bacterization with isolate 9704 and sprayed with 443 ppm 398.29 dose of Kresoxim-methyl expressed increase in number of forks. An increase of 108.38 and 100.92% in number of forks was measured using WinRHIZO.

Table 4.4(a): - Comparison of bio-regulatory effects of the Kresoxim-methyl only and Kresoxim-methyl + seed bacterization affecting root length of rice analysed using WinRHIZO

		Doses of Kresoxim-methyl (44.3% SC)*			
Trait	**Control**	**F14**	**F16**	**F18**	**F20**
Root Length (cm)	549.05	757.61	709.45	895.87	941.02
Increase over control (%)	0.00	37.98	29.21	63.17	71.39
	Control	**F14+9704**	**F16+9704**	**F18+9704**	**F20+9704**
Root Length (cm)	549.05	956.11	870.52	1240.57	1128.67
Increase over control (%)	0.00	74.14	58.55	125.95	105.57
	Control	**F14+9829**	**F16+9829**	**F18+9829**	**F20+9829**
Root Length (cm)	549.05	746.53	844.04	1156.62	1138.96
Increase over control (%)	0.00	35.97	53.73	110.66	107.44

***F14=**310.17 ppm(14µl/20ml of SDW), **F16=**354.48 ppm (16µl/20ml of SDW), **F18=**398.29 ppm(18µl/20ml of SDW) and **F20=**443 ppm (20µl/20ml of SDW).

Table 4.4 (b): - Comparison of bio-regulatory effects of the Kresoxim-methyl only and Kresoxim-methyl + seed bacterization affecting root volume of rice analysed using WinRHIZO

		Doses of Kresoxim-methyl (44.3% SC)*			
Trait	**Control**	**F14**	**F16**	**F18**	**F20**
Root Volume (cm^3)	0.72	0.91	1.00	1.25	1.15
Increase over control (%)	0.00	25.73	38.17	73.17	58.37
	Control	**F14+9704**	**F16+9704**	**F18+9704**	**F20+9704**
Root Volume (cm^3)	0.72	0.94	0.65	1.63	1.83
Increase over control (%)	0.00	29.32	(-)10.10	125.31	153.39
	Control	**F14+9829**	**F16+9829**	**F18+9829**	**F20+9829**
Root Volume (cm^3)	0.72	0.70	0.82	1.42	1.29
Increase over control (%)	0.00	(-) 3.87	13.69	95.71	78.84

***F14=**310.17 ppm (14µl/20ml of SDW), **F16=**354.48 ppm (16µl/20ml of SDW), **F18=**398.29 ppm (18µl/20ml of SDW) and **F20=**443 ppm (20µl/20ml of SDW).

Table 4.4 (c):- Comparison of bio-regulatory effects of the Kresoxim-methyl only and Kresoxim-methyl + seed bacterization affecting root surface area of rice analysed using WinRHIZO

		Doses of Kresoxim-methyl (44.3% SC)*			
Trait	**Control**	**F14**	**F16**	**F18**	**F20**
SurfArea (cm^2)	70.65	93.02	94.36	118.71	116.34
Increase over control (%)	0.00	31.66	33.57	68.04	64.68
	Control	**F14+9704**	**F16+9704**	**F18+9704**	**F20+9704**
SurfArea (cm^2)	70.65	105.99	84.35	159.35	161.20
Increase over Control (%)	0.00	50.02	19.40	125.56	128.18
	Control	**F14+9829**	**F16+9829**	**F18+9829**	**F20+9829**
SurfArea (cm^2)	70.65	80.75	93.36	143.43	136.03
Increase over Control (%)	0.00	14.31	32.15	103.02	92.55

***F14**=310.17 ppm(14µl/20ml of SDW), **F16**=354.48 ppm (16µl/20ml of SDW), **F18**=398.29 ppm(18µl/20ml of SDW) and **F20**=443 ppm (20µl/20ml of SDW).

Table 4.4(d):- Comparison of bio-regulatory effects of the Kresoxim-methyl only and Kresoxim-methyl + seed bacterization affecting number of root tips of rice analysed using WinRHIZO

		Doses of Kresoxim-methyl (44.3% SC)*			
Trait	**Control**	**F14**	**F16**	**F18**	**F20**
Root Tips	6243.00	7887.00	7472.00	10269.00	12058.00
Increase over control (%)	0.00	26.33	19.69	64.49	93.14
	Control	**F14+9704**	**F16+9704**	**F18+9704**	**F20+9704**
Root Tips	6243.00	8873.00	8705.00	10098.00	10551.00
Increase over control (%)	0.00	42.13	39.44	61.75	69.01
	Control	**F14+9829**	**F16+9829**	**F18+9829**	**F20+9829**
Root Tips	6243.00	6519.00	8092.00	10753.00	10786.00
Increase over control (%)	0.00	4.42	29.62	72.24	72.77

***F14**=310.17 ppm(14µl/20ml of SDW), **F16**=354.48 ppm (16µl/20ml of SDW), **F18**=398.29 ppm(18µl/20ml of SDW) and **F20**=443 ppm (20µl/20ml of SDW).

Table4.4 (e):- Comparison of bio-regulatory effects of the Kresoxim-methyl only and Kresoxim-methyl + seed bacterization affecting number of forks of rice analysed using WinRHIZO

		Doses of Kresoxim-methyl (44.3% SC)*			
Trait	**Control**	**F14**	**F16**	**F18**	**F20**
Number of Forks	10444.00	14722.00	10909.00	21988.00	22947.00
Increase over Control (%)	0.00	40.96	4.45	110.53	119.71
	Control	**F14+9704**	**F16+9704**	**F18+9704**	**F20+9704**
Number of Forks	10444.00	15076.00	13903.00	20984.00	21763.00
Increase over Control (%)	0.00	44.35	33.12	100.92	108.38
	Control	**F14+9829**	**F16+9829**	**F18+9829**	**F20+9829**
Number of Forks	10444.00	9220.00	12041.00	22231.00	18979.00
Increase over Control (%)	0.00	(-)11.72	15.29	112.86	81.72

***F14=**310.17 ppm(14µl/20ml of SDW), **F16=**354.48 ppm (16µl/20ml of SDW), **F18=**398.29 ppm(18µl/20ml of SDW) and **F20=**443 ppm (20µl/20ml of SDW).

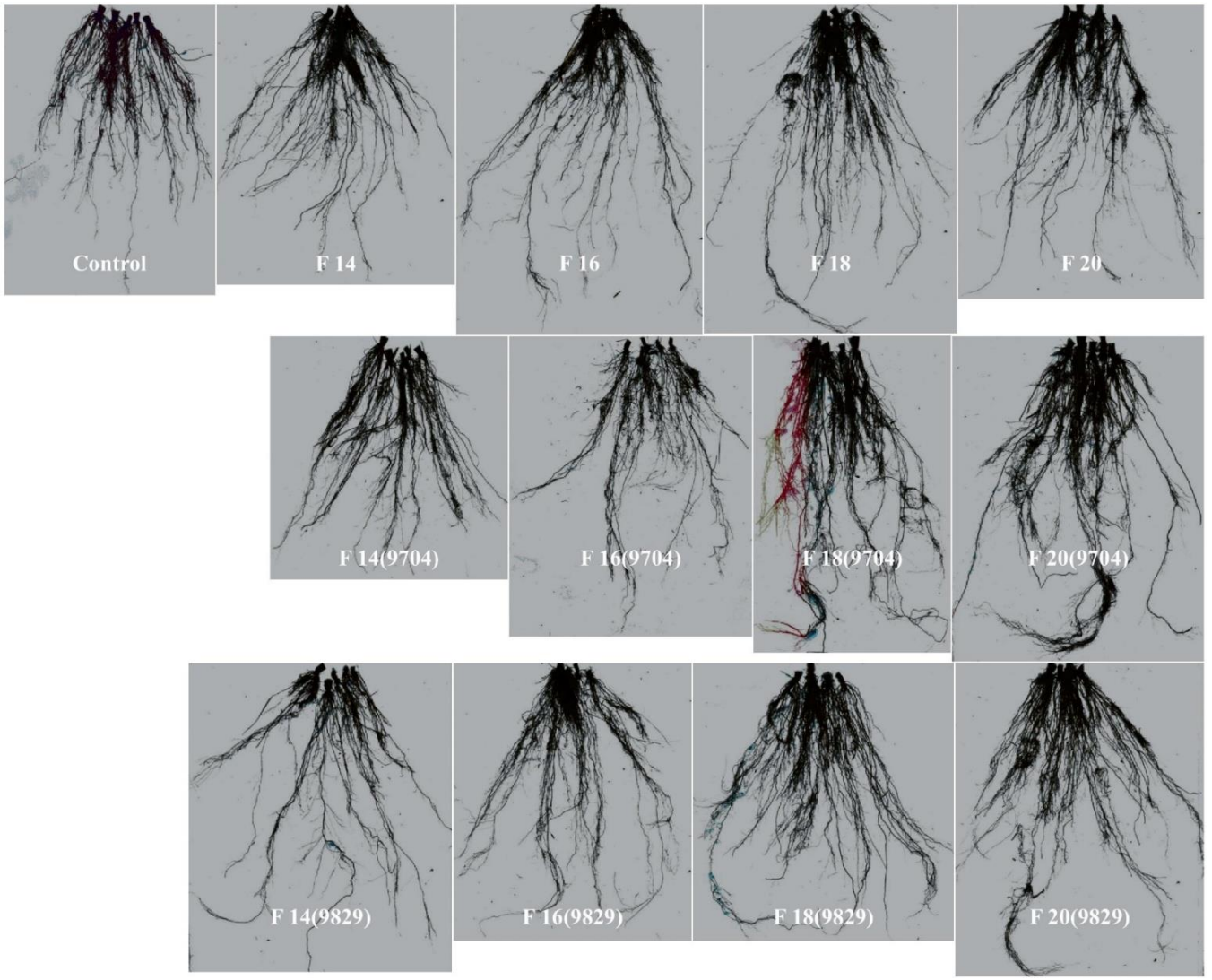


Plate4.4: Bio-regulatory effects of the fungicide Kresoxim-methylaffecting root of rice analysed using WinRHIZO

4.4 Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on chickpea

We investigated the effects of Kresoxim-methyl on total biomass and yield of chickpea and found that foliar application increased the total biomass and yield of chickpea. Significant differences in the total biomass and yield was detected in this trial as compared to control. It was observed that the total biomass and yield was increased almost in proportion to the dose of Kresoxim-methyl as compared to control (Table 4.5(a-b))

Plants derived from seed bacterization with isolates 9829 and 9704 and sprayed with 443 ppm and 398.29 ppm dose of Kresoxim-methyl expressed 49.57, 42.31% and 29.49, 29.06% increase respectively over control. Enhanced total biomass was expressed in treatments where the seed were bacterized with isolate 9704 followed by fungicide spray, as compared to the plant which were only sprayed with fungicide and also seed treatment with isolate 9829 followed by fungicide spray. A proportionate trend was observed for yield parameters in chickpea (Table 4.5(b)). Highest increase in yield (91.04%) was recorded in treatments where the seed were bacterized with isolate 9704 followed by fungicide spray, as compared to the plant which were only sprayed with fungicide and also seed treatment with isolate 9829 followed by fungicide spray.

Family Strobilurin consists of ubiquinol oxidase inhibitor (Qol) known to enhance certain morphological attributes of *Zea mays* (maize), such as increase in number of leaf and its area, and increase in shoot and root biomass (Lazo and Ascencio, 2014). Strobilurins increases the tolerance to abiotic stress, such as delay in senescence of the photosynthetic leaf, maintain the amount of the phytohormones, and increase absorption of carbon dioxide in wheat (Köhle *et al.*, 2002). The fungicide class azole also affects the physiology of plants treated with it as in case of winter wheat by rise in the chlorophyll content, delay in leaf maturity, and also protects them from abiotic stresses (Fletcher *et al.*, 2010). Movement of sedaxane from the treated seed to the rhizosphere of soil and enters inside the plant tissues which is found to initiate the root and shoot development of cereals (Swart, 2011). Previously it has been

described that wheat responds to sedaxane positively in terms of increase in biomass and growth and resistance to drought (Ajigboye *et al.,* 2016).

These morpho-physiological reactions are also known to be induced by biostimulants (Calvo *et al.,* 2014), defined as substances that at low doses are able to enhance hormone biosynthesis, nutrient uptake from the soil, resistance to biotic/abiotic stresses, crop quality, and root growth (Kauffman *et al.,* 2007). Kresoxim-methyl itself did not have auxin-like properties, nor did it stimulate auxin production, but instead it mimicked the effect of auxin by affecting other metabolic processes in the plant. Following treatment with Kresoxim-methyl, the level of 1-aminocyclopropane1-carboxylic acid (ACC), an ethylene precursor, and ACC synthase activity showed a concentration-dependent decrease and a subsequent reduction in ethylene production. Lower ethylene concentrations have been shown to slow degradation of cytokinins (Bollmark, and Eliasson, 1990), which have been shown to delay senescence (Badenoch-Jones *et al.*, 1996), and increase chlorophyll production (Kuroda *et al.,* 1996, Sundqvist 1980). The observed increases in growth, yield, and photosynthesis rate in plants treated with Kresoxim-methyl appeared to be the result of reduced chlorophyll degradation, greater assimilate concentrations, and delayed senescence. It is clear that Kresoxim-methyl has significant positive effects on plant metabolism.

Table 4.5(a):- Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting gross yield (Bundle weight) of chickpea

S. No.	Treat.*	Bundle Wt. (kg)	S. No.	Treat.	Bundle Wt. (kg)	S. No.	Treat.	Bundle Wt. (kg)
1	**F-20**	9.3^{ABC}±0.95	**5**	**9829- F-20**	10.1^{AB}±1.127	**9**	**9704- F-20**	11.667^{A}±1.202
2	**F-18**	9.333^{ABC}±1.202	**6**	**9829- F-18**	10.067^{AB}±1.157	**10**	**9704- F-18**	11.1^{AB}±0.493
3	**F-16**	9.533^{ABC}±1.235	**7**	**9829- F-16**	9.467^{ABC}±0.897	**11**	**9704- F-16**	10.667^{AB}±0.667
4	**F-14**	9.2^{BC}±0.608	**8**	**9829- F-14**	8.8^{BC}±0.702	**12**	**9704- F-14**	9.667^{ABC}±0.333
						13	**Control**	$9B^{C}$±0.577
	C.D	2.38	**SE(m)**	0.82	**SE(d)**	1.16	**C.V.**	15.099
	F value	2.147						
Percent increase over control								
1	**F-20**	19.23	**5**	**9829- F-20**	29.49	**9**	**9704- F-20**	49.57
2	**F-18**	19.66	**6**	**9829- F-18**	29.06	**10**	**9704- F-18**	42.31
3	**F-16**	22.22	**7**	**9829- F-16**	21.37	**11**	**9704- F-16**	36.75
4	**F-14**	17.95	**8**	**9829- F-14**	12.82	**12**	**9704- F-14**	23.93

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW); **F18**=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Table 4.5(b): - Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting yield of chickpea

S. No.	Treatments*	Yield (kg)	S. No.	Treatments	Yield (kg)	S. No.	Treatments	Yield (kg)
1	**F-20**	3.072^{BC}±0.283	**5**	**9829- F-20**	4.003^{AB}±0.485	**9**	**9704- F-20**	4.26^{A}±0.563
2	**F-18**	2.959^{BC}±0.057	**6**	**9829- F-18**	3.692^{AB}±0.781	**10**	**9704- F-18**	3.733^{AB}±0.598
3	**F-16**	3.487^{AB}±0.281	**7**	**9829- F-16**	3.55^{AB}±0.522	**11**	**9704- F-16**	3.547^{AB}±0.421
4	**F-14**	3.405^{AB}±0.453	**8**	**9829- F-14**	3.115^{BC}±0.266	**12**	**9704- F-14**	3.601^{AB}±0.237
						13	**Control**	2.333^{CD}±0.089
	C.D	1.071	**SE(m)**	0.369	**SE(d)**	0.522	**C.V.**	20.068
	F value	3.703						
Percent increase over control								
1	**F-20**	37.7728	**5**	**9829- F-20**	79.52167	**9**	**9704- F-20**	91.03139
2	**F-18**	32.70553	**6**	**9829- F-18**	65.54559	**10**	**9704- F-18**	67.41405
3	**F-16**	56.35277	**7**	**9829- F-16**	59.19283	**11**	**9704- F-16**	59.07324
4	**F-14**	52.67564	**8**	**9829- F-14**	39.6861	**12**	**9704- F-14**	61.46487

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW);
F18=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Bio-regulatory effects of the fungicide Kresoxim-methyl on different crops

1. Frequency of germination in chickpea, wheat and paddy was higher than control on 1st day, 3rd day and 5th day of incubation. The radicle length was larger in treated plants than control. It was also observed that radicle of germinating seeds of wheat was covered with very fine hairy roots whereas in control the radicle of germinating seeds was not covered with fine root hairs. Similarly, it was observed germination derived from paddy seed treatment with Kresoxim-methyl, the radicle of paddy seeds induced higher frequency of root hairs and lateral roots as compared to untreated control. The frequency of root hairs and lateral roots increased in radicle of germinated seedlings with the increase in dose of seed treatment Kresoxim-methyl.
2. Foliar applications of Kresoxim-methyl significantly modified morphological traits in disease-free wheat plants. Significant improvements in root length, root volume, shoot length, number of tillers and yield in wheat was measured in this trial. we found that root length, root volume, shoot length, number of tillers and yield in wheat increased almost in proportion to the dose of Kresoxim-methyl in all treatment combinations.
3. The effects of foliar application of Kresoxim-methyl in rice, and observed different morphological traits were modified in disease-free plants. The improvements in root length, root volume, shoot length detected in this trial were not significant, but we found that root length, root volume, shoot length of rice was almost in proportion to the increasing dose of Kresoxim-methyl in all treatment combinations.
4. The effect of Kresoxim-methyl on rice root morphology was analysed using WinRHIZO. Foliar application significantly modified root morphological traits in disease-free plants. Significant differences in root length, root volume, surface area, number of root tips, number of forks detected in this trial as compared to control. It was observed that root length, root volume, surface area, number of root

tips, number of forks increased almost in proportion to the dose of Kresoxim-methyl as compared to control.

5. Kresoxim-methyl foliar application increased the total biomass and yield of chickpea in disease-free plants. Significant differences in the total biomass and yield was detected in this trial as compared to control and the observed increase in the total biomass and yield almost in proportion to the dose of Kresoxim-methyl as compared to control.

In somestudies, fungicides are described to have additional effects on plant physiology. The result in improved kernel weights, protein contents correlated with a delay in the senescence of flag leaves and, grain yields due to the application of strobilurins (Qo inhibitors) has been frequently recorded. In addition to the fungicidal effect, strobilurins – which hinder fungal growth by blocking the electron transport at the cytochrome-bc complex in mitochondrial respiration (Sauter *et al*., 1995) have been reported to induce physiological changes in crops, the darker green appearance of leaves (Konradt *et al.,* 1996), like increased tolerance against abiotic stress (Köhle *et al.,* 2002), delayed senescence of photosynthetic leaf area (Wu and Von-Tiedemann 2001), increased CO – uptake (Beck, Oerke, and Dehne, 2001), and modifications in the balance of phytohormones (Grossmann and Retzlaff, 1997).

Expansion of the green leaf area duration (GLAD) triggered by strobilurins is one of the most positive side effects inscribed in the literature (Habermeyer, Gerhard, and Zinkernagel, 1998). It has been described by an effect of strobilurins on the metabolism of phytohormones. Kresoxim-methyl increased the endogenous cytokinin level of wheat tissue and reduced the 1aminocyclopropane-1-carboxylic acid (ACC) content, which catalyzes the first step in the biosynthesis of ethylene (Grossmann and Retzlaff, 1997). Various studies have investigated the effect of azole fungicides on plant physiology. Triadimefon increased the chlorophyll content of dark-adapted winter wheat by 4–40% under disease-free conditions; however, the fungicide had no effect on GLAD (Lorens and Cothren, 1989). The application of benomyl, dichlofuanid, and zineb on wheat showed in a delay of senescence compared to untreated plants (King, Jenkins, and Morgan, 1983). Estimation of the percentage

green leaf area is the main method to assess the impact of fungicides on plant senescence. This method has the limitation that the estimation of the real green leaf area is subjective and may differ among individuals. Reliable differences often have to be obvious. The use of non-destructive methods such as infrared (IR) thermography may be an alternative to establish differences in terms of plant senescence. The method gives the opportunity to have early detection of changes in canopy vitality. Near-range IR thermograph notes the temperature of plant surfaces depending on the transpiration rate. Infrared sensing of the canopy temperature has been used since the early 1980s (Grant, Chaves, and Jones, 2006), and variations in the surface temperature induced by biotic and abiotic stresses may be recorded by imaging methods (Chaerle and Straeten, 2000). Recently, numerous fungicide classes to control cereal pathogens have been developed, e.g. succinate dehydrogenase inhibitors (SDHIs), which control fungal pathogens by inhibiting the enzyme succinate dehydrogenase in the mitochondrial respiration chain of fungi (Hoersefield *et al.*, 2006). First SDHI fungicide was developed in the 1960s to manage pathogens such as *Ustilago maydis* and other basidiomycetes and were used primarily for seed dressing. The SDHIs belonging to the chemical class of pyrazole carboxamides developed recently such as penthiopyrad or bixafen are characterized to have a broader spectrum of fungicidal action including also ascomycetes of agronomic significance (Ulrich, and Matre, 1972). Bayer Crop Science detected bixafen in 2001, and it reveals high efficacy against many cereal pathogens in various field trials (Suty-Heinze *et al.*, 2011). This work aimed to examine the effects of bixafen, a pyrazole carboxamide, on the senescence and yield formation of wheat in comparison to those caused by azoles (triazolinthione), strobilurins and spiroketalamins. A series of trials was conducted under disease-free conditions in the greenhouse. Non- invasive procedures were used in order to evaluate direct effects of the fungicides on wheat plants.

4.5 Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on tolerance to water stress condition imposed on wheat plants

Strobilurin consists of ubiquinol oxidase inhibitor (Qol) known to enhance certain morphological attributes of *Zea mays* (maize), such as increase in number of

leaf and its area, and increase in shoot and root biomass (Lazo and Ascencio, 2014). Strobilurins increases the tolerance to abiotic stress, such as delay in senescence of the photosynthetic leaf, maintain the amount of the phytohormones, and increase absorption of carbon dioxide in wheat (Köhle *et al.*, 2002). The fungicide class azole also affects the physiology of plants treated with it as in case of winter wheat by rise in the chlorophyll content, delay in leaf maturity, and also protects them from abiotic stresses (Fletcher *et al.*, 2010). Movement of sedaxane from the treated seed to the rhizosphere of soil and enters inside the plant tissues which is found to initiate the root and shoot development of cereals (Swart, 2011). Previously it has been described that wheat responds to sedaxane positively in terms of increase in biomass and growth and resistance to drought (Ajigboye *et al.*, 2016).

In the present investigations, the wheat plants which were raised in pots, we investigated the effects of Kresoxim-methyl on water stress tolerance in wheat. Seeds were treated with two different isolates of fluorescent *Pseudomonas* (Isolates 9829 and 9704) and on 15 days old plant first foliar application (17-12-2019) of four increasing doses of Kresoxim-methyl (44.3% SC): 310.17 ppm (14µl/20ml of SDW), 354.48 ppm (16µl/20ml of SDW), 398.29 ppm (18µl/20ml of SDW) and 443 ppm (20µl/20ml of SDW) done. Second foliar application of four increasing doses of Kresoxim-methyl was performed on 13.01.2020. Water stress conditions were imposed to the wheat plants from 29th January till the plants started expressing leaf rolling. After 15 days (i.e. After imposing water stress on 29th January 2020) plants started showing clear difference of water stress tolerance and were greener as compared to the plants which were not sprayed with Kresoxim-methyl was not able to withstand water stress conditions. Photographs clearly depict the differences in the treatments for water stress tolerance which were taken on 22nd of February 2020.

In pots where four increasing doses of Kresoxim-methyl was only sprayed the control (unsprayed plats) were almost dry and had attained maturity where as Kresoxim-methyl sprayed plants showed “green effect”. The green effect was proportional to the four increasing doses of Kresoxim-methyl. Simlarly “green effect” condition was also observed on wheat plants which the wheat seed were bacterized with isolates 9829 and

9704 and thereafter the plants were raised. Such bacterized derived plants were sprayed with four increasing doses of Kresoxim-methyl (as detailed above). In view of the present observation, it is speculated that spray application with Kresoxim-methyl @ 398.29 ppm (18µl/20ml of SDW) and 443 ppm (20µl/20ml of SDW) alone or in combination with fluorescent *Pseudomonas* (as seed treatment) can induce water stress tolerance in wheat. Similar observation on stroburlins has been reported. Strobilurin decreases ethylene synthesis under stress and senescence conditions by attenuating the ACC synthase activity inhibiting ethylene synthesis. Ethylene is considered the plant hormone responsible for accelerating leaf senescence. This is correlated with the retardation of the fall of wheat leaves in plants treated with Kresoxim-methyl, which allows one to prolong the time of the photosynthetic activity, as the low concentrations of ethylene decrease the degradation of the cytokinins (Grossmann and Retzlaff 1997; Ypema and Gold 1999). In addition to reducing the degradation of cytokinins, strobilurins cause an increase in its synthesis, delaying the degradation of chlorophyll, causing a "green effect". As a result of diminished chlorophyll degradation, combined with better nitrogen utilization, plant senescence slows down (Grossmann *et al.,* 1999; Ruske *et al.,* 2004; Sarwat *et al.,* 2013).

Crop	**Sowing date**	**Date of spraying of four doses of Kresoxim-methyl**		**Date of water stress imposed**
		First spray	Second spray	
Wheat	04-12-2019	17-12-2019	13-01-2020	29-01-20

Plate 4.5: Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl on tolerance to water stress condition imposed on wheat plants

4.6 Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting enzyme expression in chickpea

Until recently, disease control was the only purpose of fungicide use; however, the physiological benefits of strobilurins opened a new concept for the use of these products (Venancio *et al.,* 2003). The aim of this study was to evaluate the effect Kresoxim-methyl on enzymes SOD, phenyl propanoid, lipid peroxidase, total phenolic content, PAL polyphenol oxidase contents in the leaves of chickpea plants which were derived from different treatment combinations.

1. **Effects of Kresoxim-methyl on expression of enzyme LPO (nano mole/L/g):-**

Chickpea plants derived from seed bacterization with isolate 9704 and sprayed with 443 ppm 398.29 dose and 354.48 ppm of Kresoxim-methyl expressed 187.90, 111.09 and 101.34% higher levels of LPO (nano mole/L/g). Levels of LPO content was comparatively less increase in chickpea plants derived from seed bacterization with isolate 9829 and sprayed with different doses of Kresoxim-methyl (Table 4.6 (a)), and chickpea plant which were only sprayed with different doses of Kresoxim-methyl.

2. **Effects of Kresoxim-methyl on expression of enzyme PAL (mili mole/L/g)**:-

PAL content was increased 3.125% more as compared to control in chickpea plants which were sprayed with 398.29 ppm doses of Kresoxim-methyl. Chickpea plants derived from seed bacterization with isolate 9829 and sprayed with 443 ppm 354.48 ppm dose of Kresoxim-methyl expressed 1.51 and 1.65 % higher levels of PAL (mili mole/L/g). Whereas increase levels of PAL (mili mole/L/g) (1.1406%) as compared to control was observed (Table 4.6 (b)) in chickpea plants derived from seed bacterization with isolate 9704 and sprayed with 354.48 ppm dose of Kresoxim-methyl.

3. **Effects of Kresoxim-methyl on expression of enzyme SOD (Units/g)**:-

Increased Levels of SOD (Units/g) were 1.50, 3.33 and 3.15 % higher as compared to control was measured in chickpea plates derived from seed bacterization

with isolate 9829 and sprayed with 443 ppm 398.29 ppm dose of Kresoxim-methyl and in isolate 9704 + 310.17 ppm dose of Kresoxim-methyl spray respectively. Rest of the treatment combinations expressed negative levels of SOD as compared to control. (Table 4.6 (c))

4. **Effects of Kresoxim-methyl on expression of enzyme TPC (µg GAE/g)**:-

TPC levels were increased in all the treatment combinations but it was observed that we found that the increase was not in proportion to the dose of Kresoxim-methyl in all treatment combinations. Chickpea plants receiving treatment combination of 398.29 ppm alone and in combination with isolates 9829 and 9704 expressed highest increase in TPC (µg GAE/g) (84.28, 87.19 and 96.41%) as compared to control within the respective treatment groups whereas treatment combination of 398.29 ppm with isolate 9704 expressed 96.41% increase in TPC and was the highest amongst all the treatment combinations. (Table 4.6 (d))

5. **Effects of Kresoxim-methyl on expression of enzyme Pox (ΔOD min-1g-1FW)**:-

Pox levels were variable in all the treatment combinations. Only in four treatment combination increase in Pox levels was observed as compared to control. Chickpea plants receiving treatment dose of 398.29 ppm and 310.17 ppm alone Kresoxim-methy lexpressed 7.39 and 19.85% increase in Pox as compared to control. Combination with isolates 9829 and 9704 with treatment dose of 398.29 ppm of Kresoxim-methyl expressed increased Pox level (9.13 and 8.83%) as compared to control. Proportion to the dose of Kresoxim-methyl, changes in the Pox levels were not matched in all treatment combinations.(Table 4.6 (e))

Table 4.6(a):- Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting expression of enzyme LPO (nano mole/L/g) in chickpea

S. No.	Treat*	**LPO** nano mole/L/g	S. No.	Treat.	**LPO** nano mole/L/g	S. No.	Treat.	**LPO** nano mole/L/g
1	**F-20**	9410.26^{H} ±6.41	**5**	**9829- F-20**	15112.18^{C}±144.281	**9**	**9704- F-20**	21971.16^{A}±92.713
2	**F-18**	9557.69^{H} ±44.974	**6**	**9829- F-18**	12951.93^{D}±15.625	**10**	**9704- F-18**	16108.97^{B}±70.627
3	**F-16**	8987.18^{I} ±32.187	**7**	**9829- F-16**	11980.77^{F}±173.113	**11**	**9704- F-16**	15365.38^{C}±51.285
4	**F-14**	9532.05^{H}±76.92	**8**	**9829- F-14**	10298.08^{G}±48.075	**12**	**9704- F-14**	12560.9^{E}±176.245
						13	**Control**	7631.41^{J}±15.913
				Percent increase over control				
1	**F-20**	23.31	**5**	**9829- F-20**	98.03	**9**	**9704- F-20**	187.90
2	**F-18**	25.24	**6**	**9829- F-18**	69.72	**10**	**9704- F-18**	111.09
3	**F-16**	17.77	**7**	**9829- F-16**	56.99	**11**	**9704- F-16**	101.34
4	**F-14**	24.91	**8**	**9829- F-14**	34.94	**12**	**9704- F-14**	64.59

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW);
F18=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Table 4.6(b):- Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting expression of enzyme PAL (mili mole/L/g) in chickpea

S. No.	Treat.*	**PAL** mili mole/L/g	S. No.	Treat.	**PAL** mili mole/L/g	S. No.	Treat.	**PAL** mili mole/L/g
1	**F-20**	$2.954^{E}\pm0$	**5**	**9829- F-20**	$3.096^{AB}\pm0.005$	**9**	**9704- F-20**	$2.97^{DE}\pm0.014$
2	**F-18**	$3.145^{A}\pm0.045$	**6**	**9829- F-18**	$2.916^{EF}\pm0.01$	**10**	**9704- F-18**	$3.021^{CD}\pm0.019$
3	**F-16**	$2.912^{EF}\pm0.003$	**7**	**9829- F-16**	$3.1^{AB}\pm0.009$	**11**	**9704- F-16**	$3.085^{B}\pm0.047$
4	**F-14**	$2.882^{F}\pm0.032$	**8**	**9829- F-14**	$2.959^{E}\pm0.002$	**12**	**9704- F-14**	$2.922^{EF}\pm0.017$
						13	**Control**	$3.048^{F}\pm0.050$
Percent increase over control								
1	**F-20**	(-) 3.1612	**5**	**9829- F-20**	1.5100	**9**	**9704- F-20**	(-) 2.6182
2	**F-18**	3.1255	**6**	**9829- F-18**	(-) 4.4073	**10**	**9704- F-18**	(-) 0.9363
3	**F-16**	(-) 4.5282	**7**	**9829- F-16**	1.6462	**11**	**9704- F-16**	1.1406
4	**F-14**	(-) 5.5173	**8**	**9829- F-14**	(-) 2.9961	**12**	**9704- F-14**	(-) 4.1877

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW);
F18=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Table 4.6(c):- Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting expression of enzyme SOD (Units/g) in chickpea

S. No.	Treat.*	**SOD** Units/g	S. No.	Treat.	**SOD** Units/g	S. No.	Treat.	**SOD** Units/g
1	**F-20**	3570.891^{CD}±153.551	**5**	**9829- F-20**	3814.602^{A}±52.699	**9**	**9704- F-20**	$3421.936D^{E}$±34.622
2	**F-18**	3564.775^{CD}±18.684	**6**	**9829- F-18**	3883.359^{A}±82.373	**10**	**9704- F-18**	$3597.25B^{C}$±119.592
3	**F-16**	3356.469^{E}±57.192	**7**	**9829- F-16**	$3618.247B^{C}$±29.379	**11**	**9704- F-16**	$3603.292B^{C}$±10.136
4	**F-14**	3384.457^{E}±51.733	**8**	**9829- F-14**	3319.677^{E}±20.953	**12**	**9704- F-14**	3876.531^{A}±98.696
						13	**Control**	3758.115^{AB}±20.901
				Percent increase over control				
1	**F-20**	(-) 4.98199	**5**	**9829-F-20**	1.502933	**9**	**9704- F-20**	(-) 8.94553
2	**F-18**	(-) 5.14471	**6**	**9829- F-18**	3.332495	**10**	**9704- F-18**	(-) 4.28058
3	**F-16**	(-) 10.6875	**7**	**9829- F-16**	(-) 3.72189	**11**	**9704- F-16**	(-) 4.11984
4	**F-14**	(-) 9.94282	**8**	**9829- F-14**	(-) 11.6665	**12**	**9704- F-14**	3.150807

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW);
F18=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Table 4.6(d):- Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting expression of enzyme TPC (µg GAE/g) in chickpea

S. No.	Treat.*	TPC µg GAE/g	S.No.	Treatments	TPC µg GAE/g	S. No.	Treatments	TPC µg GAE/g
1	**F-20**	154.77^{AB}±4.48	**5**	**9829- F-20**	124.965^{BC}±9.285	**9**	**9704- F-20**	127.44^{BC}±19.815
2	**F-18**	164.705^{A}±7.755	**6**	**9829- F-18**	167.315^{A}±4.735	**10**	**9704- F-18**	175.55^{A}±7.75
3	**F-16**	144.445^{ABC}±3.115	**7**	**9829- F-16**	143.515^{ABC}±5.815	**11**	**9704- F-16**	145.94^{ABC}±23.89
4	**F-14**	146.67^{ABC}±1.37	**8**	**9829- F-14**	117.48^{CD}±14.45	**12**	**9704- F-14**	163.725^{A}±6.255
						13	**Control**	89.38^{D}±1.55
Percent increase over control								
1	**F-20**	73.16	**5**	**9829- F-20**	39.82	**9**	**9704- F-20**	42.59
2	**F-18**	84.28	**6**	**9829- F-18**	87.19	**10**	**9704- F-18**	96.41
3	**F-16**	61.60	**7**	**9829- F-16**	60.57	**11**	**9704- F-16**	63.28
4	**F-14**	64.09	**8**	**9829- F-14**	31.44	**12**	**9704- F-14**	83.17

***F14**=310.17 ppm(14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW);
F18=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Table 4.6(e):- Bio-regulatory effects of the fungicide strobilurin Kresoxim-methyl affecting expression of enzyme Pox (ΔOD min-1g-1FW) in chickpea

S. No.	Treat.*	**Pox** ΔOD min-1g-1FW	S. No.	Treat.	**Pox** ΔOD min-1g-1FW	S. No.	Treat.	**Pox** ΔOD min-1g-1FW
1	**F-20**	18.67796	**5**	**9829- F-20**	35.1612597	**9**	**9704- F-20**	35.0678699
2	**F-18**	34.6009209	**6**	**9829- F-18**	17.5105875	**10**	**9704- F-18**	6.3972013
3	**F-16**	30.5384646	**7**	**9829- F-16**	26.0090593	**11**	**9704- F-16**	16.8568589
4	**F-14**	38.6166823	**8**	**9829- F-14**	29.2310074	**12**	**9704- F-14**	7.2377095
						13	**Control**	32.219481
				Percent increase over control				
1	**F-20**	(-) 42.0299	**5**	**9829- F-20**	9.128677	**9**	**9704- F-20**	8.838827
2	**F-18**	7.389574	**6**	**9829- F-18**	(-) 45.653	**10**	**9704- F-18**	(-) 80.1452
3	**F-16**	(-) 5.21892	**7**	**9829- F-16**	(-) 19.2767	**11**	**9704- F-16**	(-) 47.682
4	**F-14**	19.85314	**8**	**9829- F-14**	(-) 9.27682	**12**	**9704- F-14**	(-) 77.5366

***F14**=310.17 ppm (14µl/20ml of SDW); **F16**=354.48 ppm (16µl/20ml of SDW);
F18=398.29 ppm (18µl/20ml of SDW); **F20**=443 ppm (20µl/20ml of SDW)

Due to the large capacity of the plant to absorb these, such fungicides have positive physiological effects on the yields of crops, causing alterations in metabolism and growth (Koehle *et al.,* 2002).

The present investigation reveals that:- Variations in the levels of different enzymes analysed in the present investigation was not consistent with the increasing proportion / dose of of Kresoxim-methyl. Random combination of Kresoxim-methyl spray application induced increase in the levels of different enzymes. It was also observed that random combination of Kresoxim-methyl spray application induced decrease in the levels of different enzymes. In view of this inconsistent results conclusion could not be drawn which can exemplify that spray application of Kresoxim-methyl in increasing doses modifies the levels of different enzymes in appropriate proportions.

Wu and Tiedemann (2002) reported two fungicides, a strobilurin azoxystrobin (AZO), and a triazole, epoxiconazole (EPO), as foliar spray on spring for protection against ozone injury on leaves. Treated barley plants significantly increased the total leaf soluble protein content, activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) were increased by both fungicides at maximal rates of 16, 75, 51 and 144%, respectively. Guiacol-peroxidase (POX) activity was elevated by 50–110% only in AZO treated plants, this was the first report demonstrating marked enhancement of plant antioxidant enzymes and enhanced scavenging of potentially harmful O2 by fungicides as a mechanism of protecting plants against noxious oxidative stress from the environment (Wu and Tiedemann, 2002).

CHAPTER-V
SUMMARY AND CONCLUSION

Fungicides are known to produce fungicidal effects on fungal pathogens, but some fungicide such as strobilurins have been documented that strobilurins cause physiological changes in crops by increasing tolerance to abiotic stress, appearance of darker green leaves, delay in maturity of photosynthetic leaf area, maintenance of the balance of phytohormones, and increase uptake of carbon dioxide. Strobilurins (Qo inhibitors) have often been shown to lead to increased grain yields, kernel weights and protein content correlated with a delay in flag leaf senescence. One of the most positive side effects recorded is the "Green effects" caused by strobilurins. Mitochondrial respiration is inhibited by kresoxim-methyl which is a derivative of fungal secondary metabolite strobilurin (Sauter *et al*., 1995; Anke, 1997) and it was also reported in the wheat for inducing non-fungicidal physiological changes (Grossmann and Retzlaff, 1997) increasing tolerance to abiotic stress, appearance of darker green leaves, delay in maturity of photosynthetic leaf area, and favored corn production and plant biomass (Grossmann and Retzlaff, 1997). Kresoxim-methyl has also been reported to improve photosynthetic efficiency, through a bioregulatory auxin-like activity it influences the plant hormonal status. Therefore, the present investigation entitled **"Efficacy of kresoxim-methyl to induce plant growth promoting effects on chickpea and wheat"** was undertaken to investigate the effects of Kresoxim-methyl three crops Wheat, Rice and Chickpea. Experiments were conducted under field and also under disease-free conditions in the rainout shelters. Experiment were conducted to see the effect of four increasing doses of Kresoxim-methyl on germination, and radicle morphology of Wheat, Rice and Chickpea. Growth promoting effects observed on germination and radicle morphology of Wheat, Rice and Chickpea also prompted us to undertake with an argument that because kresoxim-methyl displays a similar mode of action to that of plant growth-promoting bacteria. Will the combination of application (Fluorescent *Pseudomonas* and kresoxim-methyl) have a significant positive effect on plant

metabolism resulting in growth and yield in different crops? We investigated the dual effect of fluorescent *Pseudomonas* applied through seed treatment and kresoxim-methyl as spray during the subsequent growth stages of crop on plant growth stimulation. Two fluorescent *Pseudomonas* isolates 9704 and 9829 were used. Wheat, Rice and Chickpea were sprayed with four increasing doses of Kresoxim-methyl and observations were recorded on different plant morphological parameters.

5.1 Bio-regulatory effects of the fungicide strobilurin kresoxim-methyl on germination on wheat, rice and chickpea

Seed treatment with fungicide is required to procure the pronounced net benefit like improved seedling emergence, plant vigor, plant height, and root biomass and plant through protection from soilborne and seedborne pathogens. Seed treated fungicide ensures the effective germination and/or emergence of seedlings, by protecting germinating seed immediately. Treatments with fungicides can result in increases / decreases or no change in germination percentages. In the present investigation, we observed the beneficial effects on germination and radicle morphology of wheat, rice and chickpea seedlings.

1. Differences in the radicle length were observed in seed Kresoxim-methyl treated seed on third day.
2. Radicle of germinating seeds of wheat, rice derived from paddy seed treatment with Kresoxim-methyl, induced higher frequency of root hairs and lateral roots as compared to untreated control.
3. The frequency of root hairs and lateral roots increased in radicle of germinated Rice seedlings with the increase in dose of seed treatment Kresoxim-methyl.

5.2 Bio-regulatory effects of the fungicide strobilurin kresoxim-methyl different crops

Kresoxim-methyl displays a similar mode of action to that of plant growth-promoting bacteria and has been found to induce physiological alterations in various crops which are often seen in connection with a positive influence on yield (Gold *et al*., 1995). Present investigation was undertaken with an argument that because kresoxim-methyl displays a similar mode of

action to that of plant growth-promoting bacteria. Will the combination of application (Fluorescent *Pseudomonas* and kresoxim-methyl) have a significant positive effect on plant metabolism resulting in growth and yield in different crops? Bio-regulatory effects of the fungicide strobilurin kresoxim-methyl on wheat, rice and chickpea were observed.

5.2.1 Bio-regulatory effects of the fungicide strobilurin kresoxim-methyl on wheat

It was observed that foliar application significantly modified morphological traits in disease-free plants. Significant improvements in shoot length, root volume, root length, number of tillers and yield was measured in this trial. We found that shoot length, root volume, root length, number of tillers and yield increased almost in proportion to the dose of kresoxim-methyl in all treatment combinations.

1. **Effects of kresoxim-methyl on root length of wheat**: - Plants sprayed with 443ppm dose of kresoxim-methyl expressed increase in root length amongst all treatment combination and control.

2. **Effects of kresoxim-methyl on root volume of wheat: -** Plants derived from seed bacterization with 9704and sprayed with 443ppm and 398.29 ppm dose of kresoxim-methyl expressed significantly enhanced root volume as compared to the plant which were only sprayed with fungicide.

3. **Effects of kresoxim-methyl on shoot length of wheat: -** Significant enhanced shoot length was expressed in treatments where the seed were bacterized with 9704 followed by fungicide spray, as compared to the plant which were only sprayed with fungicide and control.

4. **Effects of kresoxim-methyl on number of tillers of wheat: -** Significant enhanced number of tillers was expressed in treatments where the seed were bacterized with 9704 followed by fungicide spray, as compared to the plant which were only sprayed with fungicide and control.

5. **Effects of kresoxim-methyl on yield of wheat: -** Combination of seed bacterization followed with spray application of kresoxim-methyl did not resulted in significant differences as compared to the yield derived from plants prayed only with kresoxim-methyl.

5.2.2 Bio-regulatory effects of the fungicide strobilurinkresoxim-methyl on rice

We investigated the effects of kresoxim-methyl in rice, and found that foliar application modified morphological traits in disease-free plants. Although the improvements in root volume, root length, shoot length detected in this trial were not significant, we found that root volume, root length, shoot length almost in proportion to the dose of kresoxim-methyl. We found that shoot length, root volume, root length, number of tillers and yield increased almost in proportion to the dose of kresoxim-methyl in all treatment combinations.

1. **Effects of kresoxim-methyl on root length of rice**:- Highest increase in root length over all treatment combination and control was observed with seed treatment with 9704+443ppm kresoxim-methyl spray.
2. **Effects of kresoxim-methyl on root volume of rice:-** Plants derived from seed bacterization with 9704and sprayed with 443ppm dose of kresoxim-methyl expressed highest % increase in root volume
3. **Effects of kresoxim-methyl on shoot length of rice:-** Shoot length was not much influenced by spray application of kresoxim-methyl. Plants sprayed with 443ppm and 398.29 ppm dose of kresoxim-methyl expressed higher % increase in shoot length amongst all treatments. Enhanced shoot length was expressed in treatments where the seed were bacterized with 9704 followed by fungicide spray, as compared to the plant which were only sprayed with fungicide and control. Whereas shoot length was expressed comparatively less in treatments where the seed were bacterized with 9829 followed by fungicide spray, as compared to the plant which were only sprayed with fungicide and control.

5.2.3 Bio-regulatory effects of the fungicide strobilurinkresoxim-methyl on rice WinRHIZO

We investigated the effects of kresoxim-methyl in rice root morphology and found that foliar application significantly modified root morphological traits in disease-free plants. Significant differences in root volume, root length, surface area, number of root tips, number of forks detected in this trial as compared to control. It was observed that root length,

surface area, number of root tips, root volume, number of forks increased almost in proportion to the dose of kresoxim-methyl as compared to control. Plants derived from seed bacterization with 9704 and sprayed with 443ppm and 398.29 ppm dose of kresoxim-methyl expressed increased root volume, surface area, root length, number of root tips, number of forks.

5.2.4 Bio-regulatory effects of the fungicide strobilurinkresoxim-methyl on chickpea

We investigated the effects of kresoxim-methyl on yield and total biomass of chickpea and found that foliar application increased the yield and total biomass of chickpea in disease-free plants. Significant differences in the total biomass and yield was detected in this trial as compared to control. It was observed that the total biomass and yield was increased almost in proportion to the dose of kresoxim-methyl as compared to control.

Enhanced total biomass was expressed in treatments where the seed were bacterized with 9704 followed by fungicide spray, as compared to the plant which were only sprayed with fungicide and also seed treatment with 9829 followed by fungicide spray.

5.3 Bio-regulatory effects of the fungicide strobilurin kresoxim-methyl on tolerance to water stress condition imposed on wheat plants

1. In the present investigation wheat plants, which were raised in pots. We investigated the effects of kresoxim-methyl on water stress tolerance in wheat.
2. Plants sprayed with kresoxim-methyl expressed difference in water stress tolerance and were greener.
3. The green effect was proportional to the four increasing doses of Kresoxim-methyl. Similarly “green effect” condition was also observed on wheat plants in which the wheat seed were bacterized with 9829 and 9704 and thereafter the plants were raised.

5.3 Bio-regulatory effects of the fungicide strobilurin kresoxim-methyl affecting enzyme expression in chickpea

Purpose of fungicides until recently was to control the disease, however, use of strobilurins opened a new concept of the product as it initiates physiological benefits in plants along with the disease control (Venancio *et al*.,

2003). The aim of this study was to evaluate the effect Kresoxim-methyl on enzymes SOD, phenylpropanoid, lipid peroxidase, total phenolic content, PAL polyphenol oxidase contents in the leaves of chickpea plants which were derived from different treatment combinations.

1. Variations in the levels of different enzymes analysed in the present investigation was not consistent with the increasing proportion / dose of kresoxim-methyl. Random combination of kresoxim-methyl spray application induced increase in the levels of different enzymes.
2. It was also observed that random combination of kresoxim-methyl spray application induced decrease in the levels of different enzymes.
3. In view of this inconsistent results conclusion could not be drawn which can exemplify that spray application of kresoxim-methyl in increasing doses modifies the levels of different enzymes in appropriate proportions.

CONCLUSIONS

1. Larger differences in radicle length was observed in kresoxim-methyl treatement and frequency of root hairs and lateral roots increased in radicle of germinated seedlings with the increasing doses of seed treatment with Kresoxim-methyl.
2. Foliar application of kresoxim-methyl significantly improved morphological traits (root and shoot) in disease-free wheat, rice and chickpea plants. Increased in the morphological traits was almost in proportion to the dose of kresoxim-methyl in all treatment combinations.
3. Variations in the levels of different enzymes analysed in the present investigation was inconsistent. In view of this conclusion could not be drawn which can exemplify that spray application of kresoxim-methyl in increasing doses modifies the levels of different enzymes in appropriate proportions.

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