EFFECT OF METHODS OF ZINC APPLICATION ON ZINC USE EFFICIENCY AND YIELD OF WHEAT ON CALCAREOUS SOIL

by

Miss Mohini Patle
(Reg. No.016/080)

A thesis submitted to the
MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI - 413 722, DIST.AHMEDNAGAR
MAHARASHTRA, INDIA

In partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

SOIL SCIENCE AND AGRICULTURAL CHEMISTRY

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY
POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
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2018

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part

there of has not been submitted

by me or other person to any

other University or Institute

for a Degree or

Diploma

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Date : / /2018

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CERTIFICATE

This is to certify that the thesis entitled, "**EFFECT OF METHODS OF ZINC APPLICATION ON ZINC USE EFFICIENCY AND YIELD OF WHEAT ON CALCAREOUS SOIL**", submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra) in partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **SOIL SCIENCE AND AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bonafide research work carried out by **MISS. MOHINI PATLE** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

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Place: M.P.K.V., Rahuri **(R.S. Patil)**
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Place : M.P.K.V., Rahuri

Date : / /2018 (Mohini Patle)

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LIST OF ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
Ca	:	Calcium
$CaCO_3$	:	Calcium Carbonate
CD	:	Critical Difference
FYM	:	Farm Yard Manure
cm	:	Centimeter
Cu	:	Copper
dS m^{-1}	:	Deci Siemen per meter
EC	:	Electrical Conductivity
et al	:	Any others (et alli)
Fig	:	Figure
Fe	:	Iron
G	:	Gram
ha	:	Hectare
GRDF	:	General Recommended Dose of Fertilizer
K	:	Potassium
kg	:	Kilogram
L	:	Litre
meq	:	Milliequivalent
mg	:	Milligram
Mn	:	Manganese
N	:	Nitrogen
NS	:	Non Significant
P	:	Phosphorus
µg	:	Micro gram
ppm	:	Parts Per Million
S.E.	:	Standard Error
SO_4	:	Sulphate
viz.,	:	Videlicet (namely)
Zn	:	Zinc
ZnSB	:	Zinc Solubilising Bacteria

ABSTRACT

EFFECT OF DIFFERENT METHODS OF ZINC APPLICATION ON ZINC USE EFFICIENCY AND YIELD OF WHEAT ON CALCAREOUS SOIL

by

MISS MOHINI PATLE

A candidate for the degree

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2018

Research Guide : Dr. A.G. Durgude

Department : Soil Science and Agricultural Chemistry

The present investigation was undertaken to study the effect of different method of zinc application on zinc use efficiency and yield of wheat on calcareous soil. The field experiment was conducted at PGI, Research Farm. Department of Soil Science and Agril. Chemistry, M.P.K.V., Rahuri, during the *Rabi* 2016-17.

The experiment was laid out in a randomized block design with three replication and nine treatments. The treatment comprised of T_1 : Absolute control, T_2 : General recommended dose of fertilizer 120:60:40 kg ha^{-1} N : P_2O_5:K_2O + 10 t FYM ha^{-1}, T_3 : GRDF with soil application of $ZnSO_4$ @ 20 kg ha^{-1}, T_4 : GRDF with 120 kg N through zinc coated urea + 60:40 P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM, T_5 : GRDF with soil application of cow dung slurry with $ZnSO_4$, T_6 : GRDF with soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1}, T_7 : GRDF with seed coating treatment of $ZnSO_4$ @ 20 kg ha^{-1}, T_8 : GRDF with seed coating treatment of chelated Zn EDTA and T_9: GRDF with seed treatment of zinc solubilizing bacteria + soil application of zinc sulphate @ 20 kg ha^{-1}.

The results of investigation revealed that, the application of different methods of zinc in soil was influenced the soil properties. The available nutrients N, P and K at harvest were found to be significantly increased due to treatment of GRDF + soil application of $ZnSO_4$ @ 20 kg ha^{-1} with 100 kg FYM incubated for one week and treatment of GRDF + soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1}.

Total uptake of nitrogen, phosphorus and potassium by wheat crop were found significantly higher (138, 36 and 88 $kgha^{-1}$ respectively) in treatment of application of $ZnSO_4$ @ 20 kg ha^{-1} incubated with FYM for one week along with GRDF however, it was at par with treatment of application of $ZnSO_4$ @ 20 kg ha^{-1} with cow dung slurry @ 500 L ha^{-1} at 30 DAS through irrigation along with GRDF.

Total uptake of iron, zinc, manganese and copper were significantly higher (5177, 570, 1889 and 192 g ha^{-1} respectively) in treatment of GRDF + application of $ZnSO_4$ @ 20 kg ha^{-1} incubated with FYM for one week.

No significant results were found in total chlorophyll content at 45 and 65 day after sowing (DAS). The Agronomic efficiency and zinc use efficiency were significantly increase (6.26 kg kg^{-1} and 5.30 %, respectively) in treatment of GRDF + soil application of $ZnSO_4$ @ 20 kg ha^{-1} incubated with FYM for one week which was followed by GRDF+ application of $ZnSO_4$ @ 20 kg ha^{-1} with cow dung slurry @ 500 L ha^{-1} at 30 DAS through irrigation and seed coating treatment of chelated Zn EDTA @ 0.5% along with GRDF.

The grain and straw yield of wheat was significantly increased (48.39 qha^{-1} and 53.52 q ha^{-1} respectively) in treatment of GRDF with soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week. Which was at par with treatment of application of $ZnSO_4$ @ 20 kg ha^{-1} with cow dung slurry @ 500 L ha^{-1} at 30 DAS through irrigation along with GRDF i.e. 47.89 q ha^{-1} grain yield and 51.06 q ha^{-1} straw yield respectively.

The higher net monetary returns were found in treatment of soil application of zinc sulphate @ 20 kg ha^{-1} incubated with FYM and through cow dung slurry treatment along with GRDF (Rs 49155/-, Rs. 47834/- respectively) however, highest B:C ratio was recorded in control treatment followed by soil application of $ZnSO_4$ @ 20 kg ha^{-1} through incubated FYM and through cow dung slurry treatment (1.99 and 1.97, respectively).

Therefore, based on above findings, soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week or soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation along with the general recommended dose of fertilizer (120:60:40 $N:P_2O_5:K_2O$ kg ha^{-1} + 10 t ha^{-1} FYM) to wheat was found beneficial for increase in total uptake of nitrogen, phosphorus, potassium, iron, zinc, manganese and copper, agronomic efficiency, zinc use efficiency, grain and straw yield of wheat as well as for higher net monetary returns in wheat grown on medium deep black calcareous soil. The aforesaid treatments are being at par with treatment of GRDF+ seed coating treatment of chelated Zn EDTA @ 0.5% in respect of increase in zinc use efficiency, yield and monetary returns.

Pages 1 to 43

1. INTRODUCTION

In India, zinc is now considered the fourth most important yield limiting nutrient after, nitrogen, phosphorus and potassium, respectively. Analysis of 256,000 soils and 25,000 plant samples from all over India showed that 48.5% of the soils and 44% of the plant samples were potentially zinc deficient and this was the most common micronutrient problem affecting crop yields in India. Deficiency of zinc has increased in southern states due to extensive use of NPK without micronutrients. Periodic assessment of soil test data also suggests that zinc deficiency in soils of India is likely to increase from 49 to 63% by the year 2025 as most of the marginal soils brought under cultivation are showing zinc deficiency. Farming families consuming their zinc deficient crop produce leads to low zinc in their blood plasma compared to those which were fed on produce received from farms fertilized with zinc regularly. Zinc supplementation is therefore essential for maintaining high zinc content in soil, seed and blood plasma of human and animals (Singh 2009).

The importance of nutrients (micro and macro) for the normal growth of crop plants are universally recognized. In under-developed and developing countries however, plant nutrition is not being used in optimal and balanced levels and as consequence the production potential of soils are frequently not being fully exploited and the application of only major plant nutrients (N, P and K) are not adequate to achieve full potential yield of crops in many agricultural systems. Zinc is an important essential element present in plant enzymatic systems. Genc *et al*. (2006) reported that zinc has vast numbers of functions in plant metabolism and consequently zinc deficiency has a multitude of effects on plant growth. Zinc deficiency is a worldwide nutritional constraint for crop production in many types of soil in the world (Sillanpaa 1982; Rengel and Graham, 1995) and particularly in cereals growing on calcareous soil (Graham *et al.,* 1992; Cakmak *et al.,* 1996a; Singh *et al.,* 2005).

Zinc has been found useful in improving yield and yield components of wheat (Cakmak *et al*., 1996; Modaihsh, 1997; Kaya *et al*., 2002 and Singh, 2004) and adequately applied zinc has been shown to improve the water use efficiency of wheat plants (Bagci *et al*., 2007). High temperature during maturation and ripening is a major stress in many wheat production areas (Gibson and Paulsen 1999), and zinc can help provide thermo-tolerance to the photosynthetic apparatus of wheat (Graham and McDonald, 2001). In general zinc application appears to improve the overall field performance of wheat plants.

Zinc efficiency can be defined as the ratio of grain yield or shoot dry matter yield produced under Zn deficiency to that produced under Zn fertilization. Response to Zn deficiency and Zn fertilization differs greatly among cereals species and genotypes of a given species. Compared to other cereal species such as rye, triticale, barley, bread and especially durum wheat cultivars posses high sensitivity to Zn deficiency. A number of mechanisms for Zn efficiency has been discussed which includes differences in root morphology, mycorrhizal infection, release of Zn-mobilizing phytosiderophores, uptake, and translocation of Zn.

The solubility of several zinc minerals decreases in the following order namely $Zn(OH)_2$ (amorphous) > $ZnCO_3$ (smithsonite) > ZnO (zincite) > Zn $(PO_4)_2.4H_2O)$ (willemite) > soil Zn > Zn Fe_2O_4 (franklinite). All of the $Zn(OH)_2$ minerals, ZnO and $ZnCO_3$ are about 105 times more soluble than soil zinc (adsorbed to solid surfaces) and would therefore makes highly suitable fertilizer sources of zinc. Zinc forms soluble complexes with chloride, phosphate, nitrate and sulphate ions, but the neutral sulphate ($ZnSO_4$) and phosphate ($ZnHPO_4$) species are the most important and contribute to the total concentration of zinc in solution. The $ZnSO_4$ complex may increase the solubility of Zn^{2+} in soils and accounts for the increased availability of zinc when acidifying fertilisers, such as ammonium sulphate $[(NH_4(SO_4)_2)]$ are used.

Zinc deficiency is common on neutral and calcareous soils, intensively cropped soils, paddy soils and poorly drained soils, sodic and saline soils, peat soils, soils with high available phosphorus and silicon, sandy soils, highly weathered acid and coarse-textured soils. Factors such as topsoil drying, subsoil, disease interactions and high cost of fertilizer also contribute to zinc deficiency. The critical soil levels for occurrence of zinc deficiency are between 0.6 and 2.0 mg zinc kg^{-1} depending on the method of extraction used. Calcareous soils (pH>7) with moderate to high organic matter content (>1.5% organic carbon) are likely to be Zn deficient due to high HCO_3 in the soil solution. A ratio of more than 1 for exchangeable Mg:Ca in soil may also indicate Zn deficiency.

In the Indian context, more than 50% of the agricultural soils is zinc-deficient. The causes for occurrence of Zn deficiencies of this magnitude are related to the introduction of high yielding varieties, neglect of application of bulky organic manures, imbalanced use of fertilizer and low Zn uptake and accumulation of Zn which depends upon the pH, soil organic matter, temperature, crop species, etc. Zn deficiency is quite widespread in the Indo-Gangetic plain and other important cereal-growing states like Punjab, Uttar Pradesh, etc. which account for almost three-fourths of the country food grain production. The total area under Zn deficiency is about 10 Mha in India and approximately 85% of rice–wheat system cropping takes place in the Indo-Gangetic plain which has calcareous soils with high pH and thus low Zn availability. Improving production from this cereal belt is therefore, vital for sustaining grain production in the country. Zinc occurs in soil as sphalerite, olivine, hornblende, augite and biotite; however, availability of Zn from these sources is guided by several factors mentioned above. Correction of Zn deficiency through addition of Zn fertilizers is a common practice. The application of 62.5 kg $ZnSO_4$ to the first crop of the cereal-based cropping system such as cotton–wheat, bajra–wheat or rice–wheat, is sufficient to meet the Zn requirement for three years or six crops. This practice is widely followed in several states such as Punjab and Haryana. However, this approach is neither economical nor environment friendly in the long run, as only 20% of the applied Zn is available for plant uptake, while the remaining gets adsorbed on soil colloids and is, therefore, rendered immobile. As only a small fraction of the applied Zn is utilized by the crop to which it is applied, Zn accumulation in agricultural soils is on the increase, which is an environmental concern. With regard to human Zn-nutrition, fortification of Zn in food is practised, but is expensive and difficult to implement in developing countries like India, Bangladesh, Nepal, etc. Development of crop plants that are efficient Zn accumulators, especially under Zn deficiency is, therefore, a potentially important endeavour for improving zinc deficiency tolerance of cereal species *vis-à-vis*, grain productivity and micronutrient quality. There is a need for selection and/or breeding of plant genotypes with higher resistance to Zn deficiency both in terms of a higher grain yield and a higher grain Zn content.(Graham *et.al*.,1992) Realization of this approach is possible in view of the large genotypic differences in Zn sensitivity among crop plants, particularly when its availability to the roots is limited (Cakmak I. *et al*.,1998; Rengel *et al.* I and II, 1995).

In Maharashtra soils, Chavan (1978) reported that konkan soils have potential to supply total zinc in the range of 30 to 125 mg kg^{-1}. The orchard soil of Maharashtra were analysed by Jadhav *et al*. (1978) and showed that the total zinc ranged from 84 to 192 mg kg^{-1}. However, Chavan and Banerjee (1980) reported 20 to 125 mg kg^{-1} total zinc in soil profile from different agroclimatic zones of Maharashtra. Patil and Shingte (1982) and More *et al*. (1984) surveyed the drought prone area of Shirur and rice growing soils of Mulsi and Marval tehsils of Pune district and reported that the soils were varied in available zinc between 0.15-2 and 0.8-1.80 mg kg^{-1}.

Zinc is an important micronutrient. Plant response to Zn deficiency occurs in terms of decrease in membrane integrity, susceptibility to heat stress, decreased synthesis of carbohydrates, cytochromes nucleotide auxin and chlorophyll. Further, Zn-containing enzymes are also inhibited, which include alcohol dehydrogenase, carbonic anhydrase, Cu-Zn-superoxide dismutase, alkaline phosphatase, phospholipase, carboxypeptidase and RNA polymerase. Depending on the zinc level, zinc deficiency status of plants can be classified as follows: less than 10 mg kg^{-1} definite zinc deficiency; between 10 and 15 mg kg^{-1} likely to be zinc deficient; more than 20 mg kg^{-1} Zn sufficient. The ratios of P:Zn and Fe:Zn in the shoot at tillering to pod initiation stage are good indicators of zinc deficiency, while leaf Zn concentration is a less reliable indicator of zinc deficiency, except in extreme cases. Leaf Zn concentration below 15 mg kg^{-1} is regarded as Zn-deficient (Marschner, 1995).

In biological systems, Zn is involved in the activity of more than 300 enzymes. In these enzymes, Zn plays either catalytic, co-catalytic or structural roles. Zinc also plays a critical role in the synthesis of proteins and metabolism of DNA and RNA. There is also increasing evidence that several zinc-containing proteins exist, which affect gene expression directly. The recommended dietary allowances for Zn are 5 mg/day for infants, 10 mg/day for children less than 10 yrs, 15 mg/day for males more than 10 yrs, 12 mg/day for females more than 10 yrs and 15 mg/day for women during pregnancy; however, these intake limits are seldom met. Consequently, Zn deficiency in humans results in a multitude of health problems such as impairment in linear growth, sexual immaturation, impairment of learning ability and immune functions and malformations in central nervous system (Welch, 2001).

In India, wheat is grown in about 31.465 thousand ha area with a productivity of 2749.9 kg ha^{-1} resulting in to a production 86526.6 MT. It is grown in various states like Maharashtra, Madhya Pradesh, Uttar Pradesh, Rajasthan, Gujrat and Haryana etc. Among various states, Uttar Pradesh ranks first in total area (9846.0 ha) and total production 22417.4 MT of wheat. In case of productivity, Dadra and Nagar Haveli stands first (4419.4 kg ha^{-1}) followed by Uttar Pradesh (4293.9 kg ha^{-1}) (Anonymous 2015).

In Maharashtra the area under wheat cultivation during 2015 is 1067.0 hectare. The productivity was 1225.9 kg ha^{-1}, resulting into a production of 1308.0 MT during 2015 (Anonymous 2015).

Cereal crops occupy a prime position in providing food for human consumption and according to Graham and Welch (1996) about 50% of the soil used for cereal production in the world contains low level of plant available zinc which reduces not only grain yield but also nutritional quality. Cereal grains are a major source of zinc intake for persons living in developing countries and zinc deficient cereal food is creating serious health problems. Seed zinc concentrations of wheat grown under zinc deficient conditions are very low (Erdal *et al*., 2002). The fact that at least 60% of cultivated soils have growth limiting problems with mineral nutrient deficiencies and toxicities, and about 50% of the world population suffers from micronutrient deficiencies make plant nutrition research a promising avenue to meeting the global demand for sufficient food production with enhanced nutritional value in this millennium (Cakmak, 2002).

Considering high requirement of zinc in wheat crop and constraints regarding low availability of zinc in soil due to poor efficiency as influenced by high pH, calcareousness, low organic carbon content in soils. Keeping this in new, the present investigation was planned on effect of methods of zinc application on zinc use efficiency and yield of wheat grown on calcareous soil,with the following specific objectives of

1. To asses the effect of different methods of zinc application on availability of nutrient in calcareous soil.
2. To study the effect of different methods of zinc application on zinc use efficiency in calcareous soil.
3. To study the effect of different methods of zinc applications on uptake of nutrients and yields of wheat.

2. REVIEW OF LITERATURE

In India, zinc is now considered the fourth most important yield limiting nutrient after, nitrogen, phosphorus and potassium, respectively. Analysis of 256,000 soils and 25,000 plant samples from all over India showed that 48.5% of the soils and 44% of the plant samples were potentially zinc deficient and that this was the most common micronutrient problem affecting crop yields in India. Now there is need to promote the use of different type of fertilizers which correct the deficiency of zinc.

Considering the facts of nutrient status and widespread deficiency here in this chapter the review was made specifically on zinc status in soil and plant, its behavior in soil and factors regulating uptake of zinc as well as yield of crops under following appropriate subheadings.

2.1 Zinc Nutrition

Broadley *et al*. (2007) reported that zinc is an important micronutrient for cellular metabolism and biological activities such as anti-oxidative defense, carbohydrate metabolism, protein synthesis and auxin metabolism.

Sadeghzadeh (2013) stated that zinc also plays a crucial role in enzyme activity; it is the only element necessary to activate all six classes of enzymes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases).

Rathore *et al*. (2013) reported that zinc is an essential co-factor for approximately 300 individual enzymes in plants.

2.2 Zinc in Soil

2.2.1 Concentration of Zinc in Soil

In general, soils containing less than 0.5 mg DTPA-extractable Zn are considered potentially Zn deficient that may respond well to Zn fertilizers (Lindsay and Norvell, 1978).

Gibson *et al*. (1999) reported that zinc deficiency is widespread in wheat grown on alkaline calcareous soils; therefore, a large population of the world as result of this also lacks adequate Zn nutrition. Low soil Zn is attributed to a number of soil and environmental factors including low soil organic matter, high soil pH, calcareousness, water logging and arid climate. Generally, the wheat growing areas of Pakistan including Multan faces high temperature stress during reproductive growth of wheat plants. Both the rate of dry matter accumulation and duration of reproductive growth are reduced with high temperature during grain development and filling.

2.2.2 Chemical Behavior of Zinc in Soil

Brar *et al*. (1976) stated that decrease in availability of zinc in submerged soils are due to the formation of insoluble franklinite ($ZnFe_2O_4$) compound (submerged soil), insoluble ZnS (intense reduced condition), insoluble $ZnCO_3$ (partial pressure of CO_2 coupled with decomposition of OM) and insoluble $Zn(OH)_2$ (alkaline pH).

Hazra *et al*. (1987) reported that more than 84% of total Zn in soils occurs as structurally lattice bound, about 13% as sesquioxide bound, 1.6% as organically complexed and about 1% as exchangeable and water soluble forms. Only a small fraction of total Zn becomes available to crop plants.

Zinc deficiency is most commonly corrected by application of zinc sulphate ($ZnSO_4.7H_2O$) as of its high solubility and low cost (Mollah *et al*., 2009 and Fageria *et al*., 2011).

Shuman (1991) reported that zinc in soils is present in different forms, such as soluble, exchangeable, component of secondary minerals, organic matter associated, coprecipitated as secondary minerals, associated with sesquioxides and as a structural part of primary minerals.

Longragan *et al*. (1993) and Yilmaz *et al*. (1997) reported that different nutrients may interact with Zn by affecting the availability of each other from soils and their status in the plant through the process of growth or absorption, distribution and utilization. These interactions may reduce or enhance plant growth as a response to Zn.

Brar *et al*. (1976) and Slamet-Loedin *et al*. (2015) reported that zinc (Zn) interacts with both the macro and other micro-nutrients that are involved in many physiological functions of plants.

2.3 Zinc in Plant

2.3.1 Effect of Zinc on Plant Growth

Chaudhary *et al*. (1970) reported that deficiencies of copper and of zinc each depressed vegetative growth and delayed maturity, at maturity, each deficiency enhanced straw yields and depressed grain yields.

Graham *et al*. (1996) about 50% of the soil used for cereal production in the world contains low level of plant available zinc which reduces not only grain yield but also nutritional quality (low in micronutrients essential for good human health).

Yilmaz *et al*. (1997) concluded that over use of P-fertilizers resulted in even lower levels of Zn in wheat grain and human diets.

Cakmak *et al*. (1997b) reported that symptoms in wheat included loss of leaves, decreased tillering ability, inhibited growth of shoot and root and more spikelet sterility.

Hacisalihoglu *et al*. (2001) defined Zn deficiency as the condition in which only insufficient Zn is available for the optimal growth that may cause dramatic reductions in wheat yield.

Alloway (2004) stated that zinc deficiency also affected proper formation of panicles in some cereal crops.

Genc *et al*. (2007) reported that induction of longer root types *viz*., nodal, primary and lateral roots with less induction of adventitious roots has been found in barley in Zn deficiency.

Das *et al*. (2013) and Hafeez *et al*. (2013) reported that zinc deficient plants show stunted growth, delayed fruit maturity, chlorosis, shortened internodes and petioles and malformed leaves.

Jain *et al*. (2013) Zn deficiency reduced the length of primary root and increased the number and length of lateral roots in crop plant.

2.3.2 Factor Affecting Plant Zinc Uptake

Rengel (1999) found that concentrations of zinc in the grain of bread wheat ranged between 4.5 and 46 mg kg^{-1}. They concluded that fertilisation of the soil with zinc sulphate could result in an increase in grain yield and a higher grain zinc content, which would be of importance to human health.

Cakmak (2009) concluded that application of Zn-containing fertilizers represents a quick and effective approach to biofortifying cereal grains with Zn, thus being an excellent complementary tool to the breeding strategy for successful biofortification of cereals with Zn. Increasing evidence is available from field trials showing that soil and/or foliar application of Zn fertilizers improves grain Zn concentration up to 2 or 3 fold.

2.4 Zinc in Human

Generally, recommended dietary allowance for Zn is around 15 mg per day (Anonymous 1989).

Zn deficiency in humans causes a wide range of health complications, including impairments in the immune system, learning ability and physical growth, and increases in mortality and infections (Hotz and Brown, 2004).

Ho (2004) reported that Zinc deficiency induces DNA damage and increases the risk of cancer occurrence.

2.5 Zinc Availability

2.5.1 Availability in Soil

Availability of zinc in soil is influenced by various factor like soil pH, soil type and soil structure which are explained by considering following points.

2.5.1.1 Calcareous soil

Rafique *et al*. (2006) reported that zinc deficiency is a widespread micronutrient disorder on calcareous soil and is considered the third most common deficient nutrient after nitrogen and phosphorous.

Zinc is also available in chelated forms, including Zn-EDTA and Zn-EDDHA. Chelated Zn when applied to calcareous soils remains soluble and available to plants considerably longer than the inorganic forms (Hagin and Tucker, 1982).

2.5.1.2 Soil pH

Doberman *et al*. (2000) reported that the poor availability of zinc caused by water logging can be due to a relatively high pH, zinc being present as the insoluble sulphide (ZnS) and elevated concentrations of ferrous, bicarbonate and phosphate ions.

Uygur *et al*. (2000) have pointed out that calcareous soils tend to have pH values of 8 or above and that under these pH conditions, iron oxides readily precipitate out and form coatings on the carbonate minerals. They showed that an increase in pH from 8 to 8.3 can double the strength of bonding of zinc to calcite but with 0.05% of iron oxide on the calcite the bonding increases 7-fold between pH 8 and 8.3.

Janssen *et al*. (2001) found that the threshold limit value to protect an earthworm species (*Enchytraeus albidus*) could be increased from 169 mg Zn kg^{-1} to 530 mg Zn kg^{-1} when the pH of the soil was raised from 4 to 7 by liming.

Erdal *et al.* (2002) stated that seed zinc concentrations of wheat grown under zinc deficient conditions are very low.

2.5.1.3 Soil Structure

Huaqi *et al*. (2002) stated that zinc deficiency symptoms are more noticeable in plants grown under dry land soils as compared to flooded soils.

Gao *et al*.(2006) stated that the Zn bioavailability was lower in dry land soils as compared to flooded rice cultivation systems, as indicated by decreased Zn concentration not only in plant tissue but also in Zn uptake and Zn harvest index.

Gao *et al.* (2012) reported that in soil, Zinc solubility and availability to plants varies between water logged soil and dry land soil.

Gao *et al. (*2012) reported that the bioavailability of zinc in soil is controlled by both absorption-desorption reactions and solubility relation.

2.5.2 Availability in Plants

Alloway *et al*. (2009) reported that the main soil factors affecting the availability of Zn to plants are low total Zn contents, high pH, high calcite and low organic matter contents and high concentrations of Na, Ca, Mg, bicarbonate and phosphate in the soil solution or in labile forms. Maize is the most susceptible cereal crop, but wheat grown on calcareous soils and lowland rice on flooded soils are also highly prone to Zn deficiency. Zinc fertilizers are used in the prevention of Zn deficiency and in the biofortification of cereal grains.

2.6 Zinc Efficiency

2.6.1 Mechanism of Zinc Efficiency

Welch *et al*. (1982) observed that zinc played a fundamental role in the stability of plant cell membranes and that unlike the case with calcium, the effect of destabilizing membranes by zinc deficiency was not easily reversed.

Graham (1983) reviewed the evidence for linkages between zinc nutrient stress and plant disease. In general terms, he concluded the role of zinc in defence mechanisms of higher plants against disease organisms was far from clear. There were several reports of zinc mitigating the symptoms of viral infections without eliminating the viral particles from the plant. In some cases, added zinc appeared to be helping the plant overcome a virus induced zinc deficiency.

Graham *et al*. (1992) reported that significant variations in zinc efficiency can be found in wheat, barley and oats. This zinc efficiency character appeared to be poorly linked to efficiency for other micronutrients, such as manganese, which suggests an independent mechanism and genetic control for zinc efficiency.

Cakmak *et al*. (1994) demonstrated that phytosiderophores released from roots under conditions of zinc deficiency enhanced the solubility and mobility of zinc by chelation from sparingly soluble zinc compounds in calcareous soils.

Dong *et al*. (1995) reported that root architecture varies among plant species and cultivars within plants species and has been implicated in influencing plant Zn availability and zinc efficiency.

Rengel *et al*. (1995) reported that thinner roots with increased root surface area may increase the availability of Zn along with other nutrients due to a more thorough exploration of the soil.

Rengel *et al*. (1995), Cakmak (2000) and Hacisalihoglu *et al*. (2003) reported that zinc efficiency is quite possibly a shoot localized trait, we are suggesting that biochemical Zn utilization could be a key player in the mechanistic basis for crop ZE (On the molecular level, it has been found that Cu/ZnSOD gene expression was higher in Zn efficient wheat genotypes compared with in efficient ones grown under low-Zn conditions.

2.6.2 Zinc Efficiency in Plants

Grewal *et al*. (1996) showed that zinc efficient wheat genotypes were less susceptible to crown rot disease in wheat caused by *Fusarium graminearum* in soils with low zinc concentrations. They suggested that growing zinc efficient genotypes and the judicious use of zinc fertilizer was a feasible way of sustaining wheat production in areas where crown rot was a problem.

Cakmak *et al*. (1997a) studied that among the cereals Zn efficiency declines in the order rye>triticale>bread, wheat>durum wheat. The differences in expression of Zn efficiency are possibly related to a greater capacity of efficient genotypes to acquire Zn from the soil compared to inefficient genotypes.

Cakmak *et al*. (1997b) studied different rye and wheat cultivars and showed that Zn efficiency was positively correlated with the activity of Cu/ZnSOD.

Neue *et al.* (1998) reported that differences in the tolerance of rice cultivars to zinc deficiency have been recognized for many years and the International Rice Research Institute (IRRI), at Los Banos in the Philippines had been selecting for zinc-efficiency since 1971. Differential responses to zinc deficiency have been attributed either to greater physiological efficiency, or to selective higher absorption from a zinc deficient medium. Rice cultivars differ in their zinc requirements. For example, cultivar M101 thrived in a zinc deficient culture solution whereas, IR26 readily developed zinc deficiency symptoms. Affinity for zinc was twice as high in the roots of M101 than in those of IR26.

Kaylayci *et al.* (1999) found zinc efficiency to range between 57% and 92% in a selection of thirty seven bread wheat and three durum wheat cultivars. When these cultivars were grown on a zinc deficient soil in a field experiment, yield increases of between 8% and 76% were found with zinc fertilization, with an overall average yield increase of around 30%.

Genc *et al.* (2002) reported that higher ZnUE observed at lower Zn level in barley genotypes.

Cakmak (2004) reported that the screening of wheat varieties for zinc efficiency (tolerance to zinc deficiency) on zinc deficient calcareous soils in Central Anatolia, Turkey, had shown a wide range of variation in tolerance, especially among bread wheat. The most zinc efficient cultivars were those which had been developed from crosses with local landraces. Anatolian bread wheat landraces are very tolerant to zinc deficiency.

Hacisalihoglu *et al.* (2003) reported zinc enters the plant from the soil through membrane bound transporters. These transporters are involved in absorption of Zn from the soil, transport within the plant, xylem loading and unloading.

Shukla *et al.* (2014) reported that the rate of Zn uptake depends on uptake efficiency of the root system, Zn concentration at the root surface and permeability of the cell membrane.

2.7 Zinc Application

The role of zinc application on the zinc use efficiency and yield of wheat is considered as under.

2.7.1 Effect of Zinc Application on Zinc Use Efficiency

Foliar applications of micronutrients sprays have been effective towards both goals (Wilhelm *et al.*, 1988; Savithri *et al.*, 1999), but this method is too expensive to be widely practised by resource-poor farmers in some regions because of the amount of fertilizer, equipment and labour required for repeated spraying. Likewise, the difficulty in obtaining high quality micronutrient fertilizers and spreading them evenly on the soil can be prohibitive. Treating seeds with micronutrients potentially provides a simple inexpensive method for improving micronutrient plant nutrition.

Prasad *et al.* (1993) reported that soil application has the advantage of leaving residual effects on succeeding crop and thus, permitting a better utilization of applied Zn in a cropping system.

Yilmaz *et al*. (1997) concluded that soil applied Zn was a superior fertilization method compared with Zn treated wheat seed or foliar Zn applications.

Math *et al*. (2000) described that increasing application of Zn upto 50 kg zinc sulphate ha^{-1} increased the content and uptake of Zn in wheat crop.

Slaton *et al.* (2001) showed that treatments of rice seeds with Zn greatly increased grain yield and concluded that this type of Zn application method is a very economical alternative to more expensive broadcast Zn fertilizer applications.

Hacisalihoglu *et al*.(2003) reported that, biochemical Zn utilization, including the ability to maintain the activity of Zn requiring enzymes in response to Zn deficiency may be a key component of zinc efficiency. The next logical step in investigations of this trait is research on the genetic and molecular basis for zinc efficiency, in order to better understand the relationship between Zn utilization and zinc efficiency, and to identify the gene(s) controlling zinc efficiency. Progress in this research area could provide the knowledge to facilitate the engineering of Zn efficient plant varieties, which could help both crop productions on marginal soils as well as possibly improve the micronutrient density of food crops to help address significant human nutrition problems related to micronutrient deficiency.

Hacisalihoglu *et al*. (2003) showed that, in wheat, zinc efficiency (ZE) was correlated with enhanced expression and activity of zinc requiring enzymes including Cu/Zn superoxide dismutase and carbonic anhydrase. Zinc efficient genotypes may be able to maintain the functioning of these and other zinc requiring enzymes under conditions of low zinc supply. They did not find any link between zinc efficiency and root uptake of zinc nor with the translocation of zinc from the root to the shoot.

Seed treatment is a better option from an economical perspective as less micronutrient is needed, it is easy to apply and seedling growth is improved (Singh *et al.*, 2005).

Jiang *et al*. (2007) investigated the uptake and distribution of root and foliar-applied radio labelled zinc (65Zn) in aerobic rice after flowering. They found that in rice plants grown under sufficient or surplus zinc, most of the zinc accumulated in the grains originated from uptake by the roots after flowering and not from remobilization from the leaves. This suggests that root applied zinc fertilizers are likely to be more effective for the biofortification of rice grains than foliar sprays.

2.7.2 Effect of Zinc Application on Yield of Wheat

Sachdev *et al*. (1988) reported that higher yield due to zinc fertilization is attributed to its involvement in many metallic enzyme system, regulatory functions and auxin production.

Gibson *et al*. (1999) high temperature during maturation and ripening is a major stress in many wheat production areas.

Flintham *et al*. (1997) stated that optimum plant height of semi-dwarf wheat varieties ranges between 70 and 100 cm and zinc application increased plant height from 76.93 to 85.13 cm and this correlated with the increase in grain yield.

Graham *et al*. (2001) reported that zinc can help provide thermo-tolerance to the photosynthetic apparatus of wheat.

Imtiaz *et al*. (2003) reported that zinc deficiency has been reported to cause stunted plant growth and as shown here, the impact of zinc stress on wheat growth in zinc-deficient calcareous soil can be mitigated by zinc fertilization.

Imtiaz *et al.* (2003) and Ozkutlu *et al*. (2006) reported that zinc has been reported elsewhere as being effective in increasing dry matter production in wheat plants.

Jena *et al.* (2006) stated that higher concentration of zinc concentration in the grain maintained by the application of zinc in the rhizosphere with constant supply coupled with more number of productive tillers per hill and higher zinc uptake might have increased the grain yield.

Bagci *et al*. (2007) stated that zinc has been found useful in improving yield and yield components of wheat and adequately applied zinc has been shown to improve the water use efficiency of wheat plants.

Khan *et al*. (2007) reported that Zn application, significantly affected wheat grain yield, ranged from 2.7 to 3.5 t ha^{-1}, giving highest increase of 31.6% over control from 5 kg Zn ha^{-1}.

Pedda Babu *et al*. (2007) stated that zinc fertilization enhanced synthesis of carbohydrates and their transport to the site of grain production.

3. MATERIAL AND METHODS

The present investigation was carried out during the year 2016-17 in the Department of Soil Science and Agricultural Chemistry, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri on "Effect of methods of zinc application on zinc use efficiency and yield of wheat grown on calcareous soil" for enhancing growth and yield of wheat and increasing the availability of zinc to wheat by different method of zinc application through different sources of zinc. The materials used in the study, the procedures and the techniques which were adopted are given in this chapter.

In this experiment, the seed of wheat was coated with a culture of zinc solubilizing bacteria (*viz.*,*Bacillus polymyxa, Bacillus megaterium, Pseudomonas fluroscense, Glucanocetobacter diazotrophicus, Pseudomonas strita*).This culture of zinc solubilizing bacteria was obtained from the Vasantdada Sugar Institute, Manjari, Dist. Pune. The details of the materials and methods used in the present investigation are given in this chapter.

3.1 Material

3.1.1 Experimental Site

The experimental plot situated at $19^{0}34'$ N latitude and $74^{0}64'$ E longitude, medium deep black soil belonging to Inceptisol order. The soils were deficient in Zn and Fe, almost levelled, uniform in fertility status was selected for conduct of experiment.

3.1.2 Climate

The field experimental site climatically belongs to semi arid zone with an average rainfall of 519 mm. The meteorological data with respect to temperature, humidity and rainfall was obtained from the Chief Scientist, All India Coordinated Research Project on Water Management, MPKV, Rahuri and presented in table 3.1. The minimum and maximum temperature during growing period of wheat crop was $26.5^{0}C$ to $40.5^{0}C$ and 8.5 to $21.5^{0}C$ respectively. The relative humidity during the crop growth period was 36.14 to 87.29 and 13.57 to 53.28 per cent in morning and evening , respectively. Total rainfall during *rabi* crop growing period was only 15.8 mm. Sowing was done on 18^{th} November, 2016.

3.1.3 Experimental Soil

Experiment was conducted on medium deep black soil belonging Masala soil series of order Inceptisol (*Vertic Haplustepts*) having clay in texture, slightly alkaline (pH 8.18), normal in soluble salt (EC 0.34 dSm^{-1}), high in organic carbon (0.68%) and calcareous in nature (12.75% $CaCO_3$). Fertility status was low in available N (169.15 kg ha^{-1}), medium in P (17.90 $kgha^{-1}$) and high in available K (337.63 kg ha^{-1}) however, DTPA micronutrients Mn and Cu were sufficient and deficient in Fe and Zn in soil.

Table 3.1 Meteorological Weekly Mean Weather Data During October, 2016 to March, 2017

Met. Week	Temperature (^0C)		Relative humidity (%)		Sun shine (hrs)	Wind velocity ($kmhr^{-1}$)	Rainfall (mm)
	Max.	Min.	Morn.	Even.			
October, 2016							
40	28.6	21.5	87.29	71.43	2.8	3.9	15.8
41	31.6	20.6	80.43	53.28	0.7	7.1	0
42	31.8	17.2	66.57	39.00	0.4	8.1	0
43	31.3	16.4	65.86	35.57	0.3	9.1	0
44	30.6	13.9	52.33	46.33	0.4	9.6	0
November, 2016							
45	29.6	11.6	52.14	25.57	0.4	9.5	0
46	29.4	12.6	64.28	41.57	0.4	7.5	0
47	29.1	10.1	56.00	27.14	0.6	9.3	0
48	30.9	10.5	62.00	26.00	0.5	11.0	0
December, 2016							
49	28.9	11.2	64.86	34.14	1.0	8.6	0
50	28.6	11.7	52.57	37.71	1.1	7.8	0
51	29.2	10.2	54.28	34.28	0.3	9.3	0
52	29.4	8.6	56.28	28.22	0.4	9.5	0
January, 2017							
1	26.5	08.9	60.57	34.00	1.0	9.4	0
2	28.8	13.5	68.14	39.28	1.0	6.7	0
3	31.0	13.0	61.86	30.00	1.1	9.4	0
4	30.3	11.9	62.33	31.00	1.2	10.0	0
5	32.0	13.2	57.71	29.14	1.1	9.7	0
February, 2017							
6	31.4	14.0	57.28	29.71	1.1	9.5	0
7	33.6	14.3	49.43	25.43	2.2	10.2	0
8	34.4	12.7	41.86	14.42	1.6	10.7	0
9	34.2	14.8	47.00	18.90	1.3	9.1	0
March, 2017							
10	32.0	12.5	36.14	18.85	2.5	9.3	0
11	34.4	16.0	37.85	17.85	2.5	9.1	0
12	38.0	18.9	38.00	13.57	1.7	9.2	0
13	40.5	21.2	45.67	14.67	1.4	9.2	0

Table 3.2 Initial Fertility Status of Soil

Sr. No.	Parameters	Value
A.	**Chemical properties**	
1.	pH (1:2.5)	8.18
2.	EC (dS m^{-1})	0.34
3.	Organic carbon (%)	0.68
4.	Calcium carbonate (%)	12.75
5.	Available N (kg ha^{-1})	169.15
6.	Available P (kg ha^{-1})	17.90
7.	Available K (kg ha^{-1})	337.63
8.	Available Fe (µg g^{-1})	3.63
9.	Available Zn (µg g^{-1})	0.52
10.	Available Mn (µg g^{-1})	14.68
11.	Available Cu (µg g^{-1})	0.84

3.2 Details of Field Experiment

3.2.1 Layout and Experimental Design

The representative soil samples were collected plot wise for assessing the initial soil fertility status of experimental plot. The experiment was laid out in a randomized block design (Fig.1) with 9 treatments and 3 replications. The gross plot size was 3.6 m. x 3.2 m. and net plot size was 3.15 m. x 3 m. The recommended spacing of 22.5 cm was adopted.

The general recommended fertilizer dose of wheat was 120:60:40 kg ha^{-1} N, P_2O_5 and K_2O, respectively along with FYM @10 t ha^{-1}. All the nutrients and FYM were added in soils as per treatment except T_1.

3.2.2 Experimental Details

A) Experimental Details

1.	**Location**	**:**	Department of Soil Science and Agril. Chemistry, PGI Farm, M.P.K.V., Rahuri.
2.	**Crop**	**:**	Wheat
3.	**Soil type**	**:**	Medium deep black soil (Vertic Haplustepts) Inceptisol order (deficient in Zn)
4.	**Season**	**:**	*Rabi* 2016
5.	**Variety**	**:**	Samadhan (seed rate 100 kg ha^{-1})
6.	**Treatments**	**:**	9
7.	**Replications**	**:**	3
8.	**Design**	**:**	RBD
9.	**Spacing**	**:**	22.5 cm
10.	**Plot size**	**:**	Gross: 3.6 m X 3.2 m. Net : 3.15 m X 3.0 m.

B) Treatments Details

T_1 : Absolute control

T_2 : GRDF (120:60:40 N:P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM)

T_3 : T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}

T_4 : 120 kg N through zinc coated urea+60:40 P_2O_5:K_2O kg ha^{-1}+10 t ha^{-1} FYM

T_5 : T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500Lha^{-1} at 30 DAS through irrigation

T_6 : T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week

T_7 : T_2 + Seed coating treatment of $ZnSO_4$ @ 2%

T_8 : T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%

T_9 : T_2 + Seed treatment of Zinc solubilizing bacteria @ 5% + soil application of zinc sulphate @ 20 kg ha^{-1}

Note: 1) $ZnSO_4$ + cow dung slurry incubated for one week and was applied 30 DAS through irrigation.(cow dung: water was 1:4 ratio)

2) Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week and was applied at the time of sowing.

3) Treatment T_4 considering zinc content 2% in zinc coated urea, the remaining zinc was compensated through $ZnSO_4$ micronutrient fertilizer.

4) Ferrous sulphate @25 kg ha^{-1} at the time of sowing as the soil was deficient in iron.

3.3 Details of Field Operations

The details of calendar of field operations were carried out during the period of field experimentation on wheat are presented in table 3.3. The field was ploughed upto 30 cm depth by tractor. One discing and two harrowing were given (Table 3.3).

The experimental gross plot size of 3.60 m length and 3.20 m width were prepared. Farm Yard Manure @ 10 t ha^{-1} with recommended dose of nutrient (120:60:40 kg ha^{-1} $N:P_2O_5:K_2O$) were applied through urea, single superphosphate and muriate of potash in all treatment except in absolute control. The good quality of wheat seed (Var. Samadhan) were dibbled at the spacing of 22.5 cm on 18th November 2016. A light irrigation was given after the sowing of wheat seed.

3.3.1 Seed

Healthy wheat seed of variety Samadhan was obtained from the Chief Scientist, Central Seed Cell, M.P.K.V., Rahuri for the experiment.

Table 3.3 Calendar of the Field Operations for Wheat during *Rabi* 2016-17

Sr. No.	Field operations	Frequency	Date
A.	**Preparatory tillage**		
1.	Ploughing	1	26-10-16
2.	Discing and harrowing	1+2	7-11-16
3.	Stubble collection of previous crop	1	8-11-16
4.	Preparation of layout	1	10-11-16
5.	Initial soil sampling	1	11-11-16
B.	**Sowing**		
1.	Sowing of wheat as per treatment	1	18-11-16
C.	**Fertilizer(NPK)**		
	Basal dose	1	18-11-16
	30 DAS	1	18-12-16
D.	**Intercultivation**		
1.	Weeding	3	19-12-16 15-1-17 10-2-17
2.	Irrigation	5	19-11-16 10-12-16 24-1-17 10-2-17 25-2-17
E.	**Plant protection**		
1.	Spraying (Quinolphos)	2	1-12-16 19-1-17
F.	**Harvesting**		
1.	Harvesting	1	8-3-17

3.3.2 Fertilizers

The recommended dose of nutrient used for 120:60:40 kg ha^{-1} N, P_2O_5 and K_2O respectively. The required P in the form of single super phosphate and N in the form of urea, K in the form of muriate of potash and Zn in the form of $ZnSO_4.7H_2O$, Zinc coated urea, Zn-EDTA and Zinc solubilizing bacteria were used as per treatment under study. Ferrous sulphate @ 25 kg ha^{-1} was applied in all the plot except T_1 at the time of sowing.

3.3.3 Seed Inoculation

The zinc solubilizing bacteria culture was taken @ 5 % to wheat seed required for T_9 treatment in a bowl and was slightly moistened with jaggery water. First zinc solubilizing bacteria culture was treated with seed T_9 treatment and then seed treatment of Azotobacter was given to wheat seed required for all plots. The seed was dried under the shade for one hour and was used for sowing.

3.3.4 Sowing

The wheat seeds were sown on November 18^{th}, 2016 with wheat seed treatment of *Azotobacter*. The wheat seeds were sown in plots with maintaining 22.5 cm spacing between the row.

3.3.5 Irrigation

Irrigation was given at an interval of 19 to 20 days upto harvest stage.

3.3.6 Plant Protection

The incidence of sucking pest like jassids, hoppers were observed during the course of investigation. Two foliar sprays of insecticide (Quinolphos) were done.

3.3.7 Harvesting

The wheat plants in net plots after full maturity were harvested carefully for grain and straw yield and uptake studies.

3.3.8 Grain and Straw Yield

Wheat grain and straw yield from each net plot was recorded at harvest.

3.4 Details of Laboratory Analysis

3.4.1 Laboratory Material

3.4.1.1 Glasswares

The necessary glassware *viz.*, beaker, conical flask, volumetric flask, pipette, glass rod, burette, funnel, measuring cylinder, digestion tubes, etc. were used for analytical work.

3.4.1.2 Equipments

The equipments *viz.,* digestion and distillation unit, hot air oven, weighing balance, grinding machine, mechanical shaker, kel plus distillation, spectrophotometer, flame photometer, atomic absorption spectrophotometer, pH meter, conductivity meter, hot plate etc. were used during the laboratory analysis.

3.4.1.3 Chemicals

High purity (AR grade) chemicals such as sulphuric acid, sodium hydroxide, hydrogen peroxide, hydrochloric acid, potassium permanganate, sodium bicarbonate, ammonium acetate, ammonium molybdate, ammonium vanadate, boric acid, phenolphthalein, methyl red, bromocresol green etc. were used.

3.4.2 Methods

3.4.2.1 Soil analysis

Representative surface soil sample of the experimental plots were collected upto 22 cm depth at harvest stage of wheat from each plot to know the fertility status of soil. The collected soil samples were dried under shade, ground in wooden mortar and pastel, sieved through 2 mm sieve and were analyzed for pH, EC, $CaCO_3$, available N, P, K and DTPA extractable micronutrients Fe, Zn, Mn, and Cu. For organic carbon soil was sieved from 0.5 mm sieve and analyzed by using standard method (Table 3.4).

3.4.2.2 Plant analysis

The plant and grain samples were collected at harvest of wheat. The samples were air dried in sunlight and then dried in oven at 70^0C till constant weight. The whole plant sample of each treatment was ground through agate mortar and pastel after oven drying. Digestion of plant and grain samples were done and used for estimation of nutrient concentration *viz.,* N, P, K and micronutrients Fe, Zn, Mn and Cu as per standard methods mentioned in table 3.4.

Uptake of Nutrient

Per cent concentration of respective nutrient multiplied by dry weight of grain and straw yield in kg ha^{-1} and gave total uptake of nutrient in q ha^{-1}.

Nutrient Use Efficiency

$$\text{Agronomic efficiency (kg kg}^{-1}\text{)} = \frac{\text{Grain Yield in kg ha}^{-1}\text{ (Fertilized)} - \text{Grain Yield in kgha}^{-1}\text{ (Controlled)}}{\text{Quantity of nutrient applied (kg ha}^{-1}\text{)}}$$

$$\text{ZnUE (\%)} = \frac{\text{Zn uptake in Zn treated plot (g ha}^{-1}\text{)} - \text{Zn uptake in control plot (g ha}^{-1}\text{)} \times 100}{\text{Amount of Zn applied (g ha}^{-1}\text{)}}$$

(Saha *et* al., 2015)

3.4.3 Statistical Analysis

The field experiment was conducted by using randomized block design. The data obtained was analysed statistically as per the methods described by Sukhatme and Panse (1985).

Table 3.4 Standard Methods Used for Analysis of Soil and Plant Samples

Sr. No.	Parameter	Methods used	Reference
A)	**Soil analysis**		
1.	pH (1:2.5)	Potentiometric	Jackson (1973)
2.	EC	Conductometry	Jackson (1973)
3.	$CaCO_3$	Acid neutralization	Alison and Moodier (1965)
4.	Organic carbon	Walkely and Black, (Wet oxidation)	Nelson and Sommer (1982)
5.	Available N	Alkaline $KMnO_4$	Subbiah and Asija (1956)
6.	Available P	0.5 M $NaHCO_3$(pH 8.5)	Olsen and Watanabe (1954)
7.	Available K	N *N* NH_4OAc Extractant	Jackson (1973)
8.	Micronutrients (DTPA Fe, Mn, Zn and Cu)	DTPA (Atomic Absorption Spectrophotometry)	Lindsay and Norvell (1978)
B)	**Plant analysis**		
9.	Total Chlorophyll	Calorimetric method	Arnon (1949)
10.	Total N	Diacid H_2SO_4:H_2O_2 (1:1)	Jackson (1973)
11.	Total P	Diacid HNO_3:$HClO_4$ (9:4)	Chapman and Pratt (1961)
12.	Total K	Diacid HNO_3:$HClO_4$ (9:4) (Flame photometry)	Chapman and Pratt (1961)
13.	Total Fe, Zn, Mn and Cu	Diacid HNO_3: $HClO_4$ (9:4) (Atomic Absorption Spectrophotometry)	Zososki and Burau (1977)

4. RESULTS AND DISCUSSION

The present investigation was undertaken at Post Graduate Institute Research Farm, Department of Soil Science and Agril. Chemistry, M.P.K.V., Rahuri during the *rabi* 2016-17 with a view to study "Effect of methods of zinc application on zinc use efficiency and yield of wheat grown on calcareous soil". The results obtained and the observations made are presented and discussed in this chapter.

4.1 Effect of Methods of Zinc Application on Soil Chemical Properties of Calcareous Soil

The data pertaining to chemical properties of soil as influenced by different treatments of zinc application for ameliorating their deficiencies in wheat on calcareous soil are presented in table 4.1 and 4.2.

4.1.1 Soil pH

The pH of calcareous soil at harvest stage showed non-significant difference. The non-significant difference of pH at the harvest may be because the application of different methods of zinc application has no significant role in modifying soil chemical properties at harvest.

Table 4.1 Effect of methods of zinc application on pH and electrical conductivity of calcareous soil

Tr. No.	Treatment	pH (1:2.5)		EC (dS m^{-1})	
		Initial	At harvest	Initial	At harvest
T_1	Absolute control	8.14	8.16	0.36	0.31
T_2	GRDF(120:60:40 N:P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM)	8.22	8.20	0.33	0.36
T_3	T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	8.14	8.15	0.30	0.32
T_4	120 kg N through zinc coated urea+60:40 P_2O_5:K_2O kg ha^{-1}+10t ha^{-1} FYM	8.16	8.11	0.30	0.31
T_5	T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation.	8.14	8.12	0.33	0.38
T_6	T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week	8.16	8.15	0.32	0.40
T_7	T_2 + Seed coating treatment of $ZnSO_4$ @ 2%	8.22	8.20	0.36	0.38
T_8	T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%	8.20	8.22	0.37	0.36
T_9	T_2 + Seed treatment of Zinc solubilizing bacteria @5%+soil application of zinc sulphate @ 20 kg ha^{-1}	8.24	8.21	0.35	0.39
	S.Em±		0.03		0.01
	CD at 5%		NS		0.04

4.1.2 Electrical Conductivity

The electrical conductivity of soil at initial stage showed normal content (0.34 dS m^{-1}) however, EC at harvest was significantly increased (0.40 dS m^{-1}) in treatment T_6 (T_2 + soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) over T_1, T_3 and T_4 treatments under study however, T_6 was at par with treatments of T_2, T_5, T_7,T_8 and T_9. Slight increased in soil EC might be due to presence of sulphate salt in zinc sulphate added with incubated FYM in soil (fig. 2).

4.1.3 Calcium Carbonate

In case of $CaCO_3$ content in soil at harvest, no significant differences were observed due to different methods of zinc application.

4.1.4 Organic Carbon

The organic carbon content of soil was high at initial (0.68%) however, at harvest stage it showed significantly higher content in treatment of T_5 and T_6 (0.69%) over T_1, T_3 and T_7 treatment. However, treatment T_5 and T_6 were at par with treatment of T_2, T_4,T_8 and T_9 treatment (fig 3). Significant organic carbon content in soil may be due to use of slurry and FYM incubated with zinc sulphate in soil. Organic matter application in any form (FYM) induced supplementation of nitrogen and other nutrients released from organic matter decomposition, as well as to improvements in soil chemical, physical, and biological properties. Similar conclusions were also reported by other investigators (Hammad *et al.,* 2007, Hegazi, 2007 and Morsy, 2002).

Table 4.2 Effect of different methods of zinc application on organic carbon and calcium carbonate content of calcareous soil

Tr. No.	Treatment	Organic carbon (%)		Calcium carbonate (%)	
		Initial	At harvest	Initial	At harvest
T_1	Absolute control	0.67	0.62	11.33	11.06
T_2	GRDF(120:60:40 N:P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM)	0.70	0.66	12.92	12.43
T_3	T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	0.67	0.64	13.00	12.68
T_4	120 kg N through zinc coated urea+60:40 P_2O_5:K_2O kg ha^{-1}+10t ha^{-1} FYM	0.69	0.66	12.92	12.93
T_5	T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation.	0.67	0.69	12.92	10.52
T_6	T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week	0.66	0.69	12.67	11.66
T_7	T_2 + Seed coating treatment of $ZnSO_4$ @ 2%	0.66	0.62	13.42	13.00
T_8	T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%	0.71	0.65	12.67	12.67
T_9	T_2 + Seed treatment of Zinc solubilizing bacteria @5%+soil application of zinc sulphate @ 20 kg ha^{-1}	0.68	0.68	12.92	12.92
	S.Em±		0.01		0.17
	CD at 5%		0.04		NS

4.2 Effect of Different Methods of Zinc Application on Soil Available Nutrients in Calcareous Soil

The data in respect of soil available N, P and K as influenced by zinc application in wheat on calcareous soil at harvest stage are given in table 4.3 and depicted in fig.4.

4.2.1 Soil Available N, P and K

The results showed low status (169.15 kg ha^{-1}) in available nitrogen in soil at initial stage and significant variation at harvest. The available N status in soil at harvest was significantly higher (170.33 kg ha^{-1}) in application of cow dung slurry with $ZnSO_4$ treatment of T_5 over all other treatments except treatment T_6 (168.33 kg ha^{-1}).

Table 4.3 Effect of different methods of zinc application on available nitrogen, phosphorus and potassium in calcareous soil

Tr. No.	Treatment	Av. N (kg ha^{-1})		Av. P (kg ha^{-1})		Av. K (kg ha^{-1})	
		Initial	At harvest	Initial	At harvest	Initial	At harvest
T_1	Absolute control	171.00	143.33	18.00	10.43	337.67	289.33
T_2	GRDF(120:60:40 N:P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM)	168.00	154.67	16.93	13.37	338.33	309.00
T_3	T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	167.33	149.67	17.07	13.27	336.33	307.67
T_4	120 kg N through zinc coated urea+60:40 P_2O_5:K_2O kg ha^{-1}+ 10t ha^{-1} FYM	174.33	162.33	17.23	12.70	336.33	301.00
T_5	T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation.	176.33	170.33	17.37	14.20	335.00	312.00
T_6	T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week	175.33	168.33	17.33	14.63	337.00	323.00
T_7	T_2 + Seed coating treatment of $ZnSO_4$ @ 2%	168.00	159.67	17.43	14.20	339.67	317.00
T_8	T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%	167.00	157.67	17.53	12.53	335.33	308.00
T_9	T_2 + Seed treatment of Zinc solubilizing bacteria @5%+soil application of zinc sulphate @ 20 kg ha^{-1}	170.33	162.00	17.93	13.80	334.67	313.67
	S.Em±		1.58		0.12		2.02
	CD at 5%		4.76		0.37		6.07

The soil available P showed medium status (17.90 kg ha^{-1}) at initial. At harvest it was higher in treatment T_6 (14.63 kg ha^{-1}) over all other treatments. Overall available P showed low status in soil at harvest in all treatments except medium status in treatment of T_6 and T_5. The low status of available P in soil in rest of the treatment may be due to higher fixation of P in calcareous soil. Low phosphorus availability in calcareous soil might be due to their transformation to more complicated forms with $CaCO_3$ and these changed forms are rendered less available to growing plants. Similar results were also recorded by Bashour *et al.* (1983). The effect of low P solubility in alkaline and calcareous soil was due to poor fertilizer P efficiency. The similar results were also supported by Stark and Westermann (2003) and Javid and Rowell (2003).

Soil available K status was high (337.63 kg ha^{-1}) in soil at initial stage but the available K status at harvest showed significantly higher status in T_6 treatment over all other treatment except T_7. The available K status at harvest stage showed very high fertility status which did not change the category of fertility status of available K in soil as influenced by various treatments.

4.3 Effect of Different Methods of Zinc Application on Available Zinc and Iron in Calcareous Soil

The data in respect of soil available Zn and Fe content in soil at initial and at harvest stage as influenced by different methods of zinc application to wheat grown on calcareous soil are given in table 4.4 and depicted in fig.5

Table 4.4 Available zinc and iron content in soil as influenced by methods of zinc application in calcareous soil

Tr. No.	Treatment	DTPA-Zn (µg g^{-1})		DTPA-Fe (µg g^{-1})	
		Initial	At harvest	Initial	At harvest
T_1	Absolute control	0.54	0.44	3.59	2.81
T_2	GRDF(120:60:40 N:P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM)	0.51	0.47	3.57	2.89
T_3	T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	0.52	0.56	3.63	3.03
T_4	120 kg N through zinc coated urea+60:40 P_2O_5:K_2O kg ha^{-1}+10t ha^{-1} FYM	0.52	0.54	3.61	2.95
T_5	T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation.	0.54	0.58	3.60	3.13
T_6	T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week	0.51	0.59	3.77	3.57
T_7	T_2 + Seed coating treatment of $ZnSO_4$ @ 2%	0.52	0.48	3.69	3.52
T_8	T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%	0.50	0.47	3.59	3.46
T_9	T_2 + Seed treatment of Zinc solubilizing bacteria @5%+soil application of zinc sulphate @ 20 kg ha^{-1}	0.51	0.49	3.63	3.48
	S.Em±		0.01		0.25
	CD at 5%		0.03		NS

4.3.1 DTPA Iron and Zinc

The soils were deficient in iron and zinc as the critical limit of iron and zinc are 4.5 and 0.6 µg g^{-1} respectively. The results showed low status (3.63 µg g^{-1}) at initial stage as well as at harvest (3.31 µg g^{-1}) in respect of soil available iron but in case of available zinc, the result showed low status(0.52 µg g^{-1})at initial but at harvest available Zn was found to be significantly increased in T_6 treatment (0.59 µg g^{-1}) over all other treatment except T_3 (0.56 µg g^{-1}) and T_5

(0.58 µg g^{-1}). This might be due to natural chelates of humic and fulvic acid formed during incubation and slurry treatment may be chelates with the zinc and its efficiency increased in soil, reflected in increased of available zinc in soil at harvest.

4.4 Effect of Different Methods of Zinc Application on Available Copper and Manganese in Calcareous Soil

The data on soil available copper and manganese as influenced by different methods of zinc application has been given in table 4.5 and depicted in fig.5

4.4.1 DTPA Copper and Manganese

The result showed sufficient (14.68 µg g^{-1}) in available manganese at initial stage and at harvest, available Mn was found to be significantly higher in T_6 treatment (14.03 µg g^{-1}) over all other treatment except T_4 (13.82 µg g^{-1}) and T_5 (13.80 µg g^{-1}). This is because of production of root exudates that are toxic to Mn-oxidizing microorganisms in the rhizosphere which increased Mn availability. Similar results were also reported by Timonin (1946).

Table 4.5 Available copper and manganese content in soil as influenced by methods of zinc application in calcareous soil

Tr. No.	Treatment	DTPA-Mn (µg g^{-1})		DTPA-Cu (µg g^{-1})	
		Initial	At harvest	Initial	At harvest
T_1	Absolute control	14.50	12.18	0.82	0.62
T_2	GRDF(120:60:40 N:P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM)	14.89	13.56	0.86	0.73
T_3	T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	14.98	13.58	0.84	0.80
T_4	120 kg N through zinc coated urea+60:40 P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM	14.51	13.82	0.86	0.76
T_5	T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation.	14.45	13.80	0.85	0.72
T_6	T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week	14.95	14.03	0.82	0.89
T_7	T_2 + Seed coating treatment of $ZnSO_4$ @ 2%	14.91	13.53	0.84	0.76
T_8	T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%	14.69	13.26	0.86	0.80
T_9	T_2 + Seed treatment of Zinc solubilizing bacteria @5%+soil application of zinc sulphate @ 20 kg ha^{-1}	14.29	13.36	0.82	0.74
	S.Em±		0.09		0.01
	CD at 5%		0.28		0.03

Similarly in case of copper at initial result was sufficient (0.84 µg g^{-1}) but at harvest it was significantly higher in treatment T_6 (0.89 µg g^{-1}) over all other treatments. So this may be due to application of zinc fertilizer which enable Zn^{2+} activity much higher than Cu^{2+} activity at the absorbing sites making it an effective competitor in Cu absorption and making its absorption less sensitive to competition from Cu. Similar result also showed by Loneragan *et al*. (1993).

4.5 Effect of Methods of Zinc Application on Total Chlorophyll Content

Total chlorophyll content showed non-significant difference among the treatments with highest being in treatment T_6 (1.58 mg g^{-1}at 45 DAS and 1.70 mg g^{-1} at 65 DAS). This may be because of iron uptake which is essential for chlorophyll synthesis. Increased chlorophyll contents are due to zinc which acts as a structural and catalytic component of proteins, enzymes and as co-factor for normal development of pigment biosynthesis. Similar results were also reported by Balashouri (1995).

Table 4.6 Total chlorophyll content as influenced by methods of zinc application on calcareous soil

Tr. No.	Treatment	Total chlorophyll ($mg\ g^{-1}$ fresh plant tissue)	
		At 45 DAS	At 65 DAS
T_1	Absolute control	1.35	1.55
T_2	GRDF(120:60:40 $N:P_2O_5:K_2O$ kg ha^{-1} + 10 t ha^{-1} FYM)	1.41	1.68
T_3	T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	1.46	1.65
T_4	120 kg N through zinc coated urea+60:40 $P_2O_5:K_2O$ kg ha^{-1} +10t ha^{-1} FYM	1.42	1.75
T_5	T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation.	1.37	1.66
T_6	T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week	1.58	1.70
T_7	T_2 + Seed coating treatment of $ZnSO_4$ @ 2%	1.39	1.61
T_8	T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%	1.52	1.63
T_9	T_2 + Seed treatment of Zinc solubilizing bacteria @5%+soil application of zinc sulphate @ 20 kg ha^{-1}	1.38	1.59
	S.Em±	0.10	0.09
	CD at 5%	NS	NS

4.6 Effect of Different Methods of Zinc Application on Total Uptake of Nutrients in Wheat Grown on Calcareous Soil at Harvest Stage

The observation in respect of total uptake of N, P and K by wheat as influenced by effect of methods of zinc application to wheat on calcareous soil are presented in table 4.7 and depicted in fig.6

4.6.1 Total Uptake of Nitrogen

The data in respect of total nitrogen uptake by wheat was found significant results as influenced by methods of zinc application in calcareous soil. The treatments of T_5 and T_6 showed significantly higher uptake of total N (138 kg ha^{-1}) as compare to all other treatments except T_4 (135 kg ha^{-1}) and T_9 (134 kg ha^{-1}) which were at par with treatment T_5 (138 kg ha^{-1}) and T_6 (138 kg ha^{-1}). Higher total uptake of nitrogen was may be due to application of zinc sulphate @ 20 kg ha^{-1} incubated one week with FYM and use of cow dung slurry given through irrigation might have increased the zinc use efficiency in soil as compare to other treatments. Potarzycki and Grzebisz (2009) also reported similar result that zinc exerts a great influence on basic plant life processes such as nitrogen metabolism and uptake of nitrogen.

Table 4.7 Effect of methods of zinc application on total uptake of n, p and k by wheat crop at harvest stage

Tr. No.	Treatment	Total uptake of macronutrient (kg ha^{-1})		
		N	P	K
T_1	Absolute control	101	29	72
T_2	GRDF (120:60:40 N:P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM)	125	33	75
T_3	T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	127	32	79
T_4	120 kg N through zinc coated urea + 60:40 P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM	135	34	78
T_5	T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation.	138	34	85
T_6	T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week	138	36	88
T_7	T_2 + Seed coating treatment of $ZnSO_4$ @ 2%	131	31	78
T_8	T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%	129	33	75
T_9	T_2 + Seed treatment of Zinc solubilizing bacteria @ 5% + soil application of zinc sulphate @ 20 kg ha^{-1}	134	30	82
	S.Em±	1.38	1.35	1.40
	CD at 5%	4.14	4.05	4.21

4.6.2 Total Uptake of Phosphorus

The highest total P uptake by wheat plant was found significantly higher in treatment of T_6 (36 kg ha^{-1}) over treatments of T_1, T_7 and T_9, however, treatment T_2, T_3, T_4, T_5 and T_8 were at par with treatment T_6. This can be because of application of zinc sulphate @ 20 kg ha^{-1} along with incubated FYM for one week might have increased the availability of P in soil.

4.6.3 Total Uptake of Potassium

Data on total potassium uptake by wheat revealed that, the significant increased in total K uptake (88 kg ha^{-1}) was observed in treatment T_6 (T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) over all other treatments except treatment T_5 (85 kg ha^{-1}) which was at par with T_6.

The higher total uptake of nutrients (N, P and K) might be due to increased in grain and stover yield of wheat and availability of zinc in soil due to incubation and slurry treatment. The increase in total N and K uptake could be attributed to synergistic effect between N and Zn and due to the positive interaction of K and Zn, respectively. The present findings also support the results of Ashoka *et al*. (2008), Morshedi and Farahbakhsh (2010).

4.7 Effect of Different Methods of Zinc Application on Total Uptake of Iron and Zinc by Wheat Grown on Calcareous Soil at Harvest Stage

The data in respect of total uptake of iron and zinc as influenced by zinc application to wheat on calcareous soil are given in table 4.8 and depicted in fig.7

The results indicated that, there was significant variation in uptake of Fe and Zn. The maximum total micronutrient uptake Fe (5177 g ha^{-1}), and Zn (570 g ha^{-1}), were found to be significantly higher in treatment T_6 (T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) over the rest treatments.

The increase in the zinc content in grain and straw might be due to the presence of increased amount of Zn in soil solution by the application of zinc fertilizer that facilitated greater absorption. Similar result was also reported by Sakal *et al*., 1987; Mollah *et al*., 2009; Fageria *et al*., 2011.

Higher uptake of iron probably due to differences in experimental details, especially in plant species and the concentration, ionic state and complexation of Fe. Similar results were also supported by Giordano *et al*.(1974).

4.8 Effect of Different Methods of Zinc Application on Total Uptake of Copper And Manganese in Wheat Grown on Calcareous Soil at Harvest Stage

The results indicated that, there was significant variation in uptake of Cu and Mn. The maximum total micronutrient uptake of Cu (192 g ha^{-1}) was found in T_6 (T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) over all other treatments except T_5 treatment (188 g ha^{-1}) which was at par with treatment T_6, and total uptake of Mn (1089 g ha^{-1}), was found higher in treatment T_6 (T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) over all other treatments except T_5 which was at par with treatment T_6. This might be due to exudation of phytase which is important for Mn uptake from high-pH soils, similar results also observed by George *et al*. (2014).

Table 4.8 Effect of different methods of zinc application on total uptake of iron, zinc manganese and copper by wheat crop at harvest stage

Tr. No.	Treatment	Total uptake of micronutrients (g ha^{-1})			
		Fe	Zn	Mn	Cu
T_1	Absolute control	2559	344	625	107
T_2	GRDF (120:60:40 N:P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM)	3877	397	924	132
T_3	T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	4373	474	850	138
T_4	120 kg N through zinc coated urea + 60:40 P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM	5106	429	891	170
T_5	T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation.	5176	510	1085	188
T_6	T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week	5177	570	1089	192
T_7	T_2 + Seed coating treatment of $ZnSO_4$ @ 2%	3950	386	854	113
T_8	T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%	4876	491	1014	166
T_9	T_2 + Seed treatment of Zinc solubilizing bacteria @ 5% + soil application of zinc sulphate @ 20 kg ha^{-1}	4233	440	893	175
	S.Em±	0.98	1.32	1.38	1.41
	CD at 5%	2.92	3.97	4.15	4.25

4.9 Effect of Different Methods of Zinc Application on Yield of Wheat Grown on Calcareous Soil at Harvest Stage

The data in respect of grain and straw yield as influenced by zinc application in wheat on calcareous soil are presented in table 4.9 and depicted in fig.8

4.9.1 Grain Yield

It was observed from grain yield data of wheat that, the grain yields were significantly increased (2.00 to 14.13 per cent) over general recommended dose of fertilizer treatment (T_2) with significantly highest (48.39 q ha^{-1}) in treatment of T_6 (T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) over all other treatments except treatment T_5 and T_8 (T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation and T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%) having yield of 47.89 and 46.56 q ha^{-1} respectively, which were at par with treatment T_6. Soil application of 100 kg FYM incubated with $ZnSO_4$ @ 20 kg ha^{-1} along with recommended dose of fertilizer (T_6) recorded 14.13 % more grain yield as compared to only recommended dose of treatment (T_2). This might be due to availability of zinc in soil and its uptake by wheat crop.

Table 4.9 Effect of zinc application on grain and straw yield of wheat in calcareous soil

Tr. No.	Treatments	Yield (q ha^{-1})		Percent increase yield over T_2 treatment	
		Grain	Straw	Grain	Straw
T_1	Absolute control	33.35	35.48	-	-
T_2	GRDF (120:60:40 $N:P_2O_5:K_2O$ kg ha^{-1} + 10 t ha^{-1} FYM)	42.40	41.48	-	-
T_3	T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	43.25	47.05	2.00	13.15
T_4	120 kg N through zinc coated urea + 60:40 $P_2O_5:K_2O$ kg ha^{-1}+10 t ha^{-1} FYM	44.42	47.65	4.76	14.87
T_5	T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation.	47.89	51.06	12.95	23.09
T_6	T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week	48.39	53.52	14.13	29.03
T_7	T_2 + Seed coating treatment of $ZnSO_4$ @ 2%	42.44	44.59	0.09	7.50
T_8	T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%	46.56	50.45	9.81	21.62
T_9	T_2 + Seed treatment of Zinc solubilizing bacteria @ 5% + soil application of zinc sulphate @ 20 kg ha^{-1}	44.98	48.38	6.08	16.63
	S.Em±	1.03	0.85		
	CD at 5%	3.10	2.57		

4.9.2 Straw Yield

It was observed from the straw yield of wheat data that, the per cent increased in straw yield was 7.50 to 29.03 over GRDF of treatment T_2. The treatment T_6 (T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) showed significantly higher straw yield (53.52 q ha^{-1}) among all the treatments except treatment T_5 (T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation) with straw yield of 51.06 q ha^{-1} which was at par with treatment T_6. Soil application of 100 kg FYM incubated with $ZnSO_4$ @ 20 kg ha^{-1} along with recommended dose of fertilizer (T_6) found 29.03 % more straw yield (T_6) as compared to only RDF treatment (T_2) under study. This might be due to slight increase in chlorophyll content in leaves of wheat plant and availability of zinc in soil during plant growth.

The increase in grain and straw yield due to Zn fertilization might be the fact that Zn plays an important role in biosynthesis of the IAA and initiation of primodia for reproductive parts and a result of favourable effect of zinc on the metabolic reactions within the plants. Similar results were also reported by Goswami (2007), Singh *et al.* (2012) and Keram *et al.* (2012).

4.10 Effect of Different Methods of Zinc Application on Agronomic Efficiency and Zinc Use Efficiency in Wheat Grown on Calcareous Soil

The data in respect of agronomic efficiency and zinc use efficiency as influenced by zinc application in wheat on calcareous soil are presented in table 4.10.

4.10.1 Agronomic Efficiency

Agronomic efficiency of wheat influenced by different methods of zinc application to wheat on calcareous soil are presented in table 4.10.

Table 4.10 Agronomic efficiency and zinc use efficiency of wheat as influenced by different methods of zinc application to wheat on calcareous soil

Tr. No.	Treatment	Agronomic efficiency (kg kg^{-1})	Zinc use efficiency (%)
T_1	Absolute control	-	-
T_2	GRDF(120:60:40 N:P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM)	4.11	1.26
T_3	T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	4.12	3.09
T_4	120 kg N through zinc coated urea + 60:40 P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM	5.03	2.02
T_5	T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation	6.05	3.90
T_6	T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week	6.26	5.30
T_7	T_2 + Seed coating treatment of $ZnSO_4$ @ 2%	4.13	1.00
T_8	T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%	6.00	3.50
T_9	T_2 + Seed treatment of Zinc solubilizing bacteria @5%+soil application of zinc sulphate @ 20 kg ha^{-1}	4.84	2.28
	S.Em±	0.05	0.09
	CD at 5%	0.17	0.27

Agronomic efficiency was increased in different methods of zinc application on wheat in treated plots over untreated treatment (T_1). The agronomic efficiency was significantly recorded the highest (6.26 kg kg^{-1}) in treatment T_6 (T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) over all the treatments, however it followed by treatment T_5 (6.05 kg kg^{-1}) and T_8 (6.00 kg kg^{-1}).

This indicated increase in nutrient use efficiency due to different methods of zinc application. This might be due to increase in total uptake of N, P, K and Fe, Cu. Mn and Zn by wheat plant as well as increase in availability of Zn in soil.

4.10.2 Zinc Use Efficiency

Zinc use efficiency was increased in different methods of zinc application on wheat in treated plots over control (T_1). The zinc use efficiency was recorded significantly highest (5.3 %) in treatment T_6 (T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) over all other treatments. Treatment T_3 and T_8 were significantly increased the zinc use efficiency (3.09 % and 3.50 %, respectively) over T_2 (GRDF).This might be due to increase in availability of Zn in soil and uptake of zinc by plant (fig.10).

4.10 Economics

The cost of cultivation was recorded the highest (Rs. 50679/-) in treatment T_6 (T_2 + soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) however, it was lowest (Rs. 31188/-) recorded in treatment T_1 (Absolute control). The gross monetary returns was recorded the highest (Rs. 98730/-) in treatment of T_6 followed by treatment T_5 (Rs. 97349/-). Highest net return was recorded in treatment of T_6 (Rs. 49155/-). The benefit: cost ratio was found highest (2.17) in treatment of T_1, followed by T_6 (1.99) and T_5 (1.97).

Table 4.11 Economics

Tr. No.	Treatment	Cost of cultivation	Gross monetary returns	Net monetary return	Benefit : Cost ratio
		--------Rs. ha^{-1}------			
T_1	Absolute control	31188	67778	36590	2.17
T_2	GRDF(120:60:40 N:P_2O_5:K_2O kg ha^{-1} + 10 t ha^{-1} FYM)	48455	85484	37029	1.76
T_3	T_2 + Soil application of $ZnSO_4$ @ 20 kg ha^{-1}	49455	88094	38639	1.78
T_4	120 Kg N through zinc coated urea+60:40 P_2O_5:K_2O kg ha^{-1}+10 t ha^{-1} FYM	48691	90350	41659	1.85
T_5	T_2 + Soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation.	49515	97349	47834	1.97
T_6	T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week	49575	98730	49155	1.99
T_7	T_2 + Seed coating treatment of $ZnSO_4$ @ 2%	48555	86146	37591	1.77
T_8	T_2 + Seed coating treatment of chelated Zn EDTA @ 0.5%	49090	94798	45708	1.93
T_9	T_2 + Seed treatment of Zinc solubilizing bacteria @5%+soil application of zinc sulphate @ 20 kg ha^{-1}	49460	91514	42054	1.85

Note: Rs. 13.04 kg^{-1} N, Rs. 48.75 kg^{-1} P_2O_5, Rs. 19.43 kg^{-1} K_2O, Rs. 1200 t^{-1} FYM, Rs. 1831 q^{-1} grain yield, Rs. 189 q^{-1}stover yield, Rs. 50 kg^{-1} $ZnSO_4.7H_2O$, Rs. 200 L^{-1} ZnSB, Rs 107 $100g^{-1}$ chelated Zn.

5. SUMMARY AND CONCLUSION

The present investigation was carried out to study the "Effect of different methods of zinc application on zinc use efficiency and yield of wheat grown on calcareous soil" which was conducted at Post Graduate Research Farm, Department of Soil Science and Agril. Chemistry, M.P.K.V., Rahuri during the *rabi* 2016-17.

The experimental soil type was an Inceptisol (*Vertic Haplustept*) and it content low available nitrogen and medium in phosphorus content and very high in available potassium content. However, soil was deficient in DTPA Zn and Fe The yield of wheat was estimated to evaluate the treatment effects. The total chlorophyll content, total uptake of nutrients were recorded and available N, P, K, organic carbon and calcium carbonate content of soils were estimated initially as well as at harvest of wheat.

The results obtained and reported from present investigation are summarized as follows.

5.1 Summary

5.1.1 Effect of Different Methods of Zinc Application to Wheat on Chemical Properties of Calcareous Soil

The soil pH did not show any significant differences among the treatments at harvest.

There was significant difference in soil EC at harvest, and was significantly highest in treatment of T_6 (0.40 dS m^{-1}).

In case of $CaCO_3$ content in soil there was no significant differences at harvest but in case of organic carbon content of soil showed significant difference at harvest.

5.1.2 Effect of Different Methods of Zinc Application to Wheat on Available N, P and K in Calcareous Soil

There was significant differences among the treatments in soil available nitrogen which was significantly higher in treatment T_5 (170.33 kg ha^{-1}) and treatment T_6 (168.33 kg ha^{-1}) at harvest. Similarly soil available phosphorus was significantly higher in treatment T_6 (14.63 kg ha^{-1}) and available potassium was significantly higher in treatment T_6 (323 kg ha^{-1}) at harvest as influenced by different method of zinc application to wheat.

5.1.3 Effect of Different Methods Zinc Application in Wheat on Available Iron and Zinc in Calcareous Soil

The availability of iron shows no significant differences among the treatments at harvest but in case of zinc at harvest, available zinc was found to be significantly higher in treatment T_6 (0.59 µg g^{-1}) due to increase in availability of Zn in soil and uptake of zinc by wheat plants.

5.1.4 Effect of Different Methods Zinc Application in Wheat on Available Copper and Manganese in Calcareous Soil

The availability of manganese and copper showed significant differences among the treatment at harvest, these were found to be significantly higher in treatment T_6 (14.03 µg g^{-1} and 0.89 µg g^{-1} respectively).

5.1.5 Effect of Different Methods of Zinc Application on Total Chlorophyll Content in Wheat Leaves

No significant results were found in total chlorophyll content at 45 DAS and 65 DAS.

5.1.6 Effect of Different Methods of Zinc Application on Total Macronutrient Uptake by Wheat at Harvest Stage

Total nitrogen uptake by wheat in treated plants showed higher uptake compared to the controlled one with highest being in treatments of T_5 and T_6 (138 kg ha^{-1}), where, soil application of $ZnSO_4$ @ 20 kg ha^{-1} was applied in soil by incubation with FYM and slurry of cow dung.

Total phosphorus and potassium uptake (36 and 88 kg ha^{-1} respectively) by wheat was significantly increased in treatment T_6 (T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week).

5.1.7 Effect of Different Methods of Zinc Application on Total Uptake of Iron and Zinc by Wheat at Harvest Stage

Total uptake of iron and zinc at harvest stage was the highest (5177 g ha^{-1} and 570 g ha^{-1} respectively) in treatment T_6 (T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week).

5.1.8 Effect of Different Methods of Zinc Application on Total Uptake of Copper and Manganese by Wheat at Harvest Stage

Total uptake of manganese and copper at harvest stage was highest (1089 g ha^{-1} and 192 g ha^{-1}, respectively) in treatment T_6 (T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week).

5.1.9 Effect of Different Methods Zinc Application on Yield of Wheat

The highest significant response in terms of grain (48.39 q ha^{-1}) and straw (53.52 q ha^{-1}) yield of wheat was noticed in the treatment T_6 (T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week).

5.1.10 Nutrient Use Efficiency as Influenced by Different Methods of Zinc Application in Wheat on Calcareous Soil

The agronomic efficiency was significantly recorded the highest (6.26 kg kg^{-1}) in treatment T_6 (T_2 + Soil application of 100 kg FYM +$ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) followed by treatment T_5 (6.05 kg kg^{-1}) and T_8 (6.00 kg kg^{-1}).

5.1.11 Zinc Use Efficiency as Influenced by Different Methods of Zinc Application in Wheat on Calcareous Soil

The zinc use efficiency was significantly recorded the highest (5.3 %) in treatment T_6 (T_2 + Soil application of 100 kg FYM + $ZnSO_4$ @ 20 kg ha^{-1} incubated for one week) followed by treatment T_5 (3.9 %) and T_8 (3.50 %).

5.1.12 Economics

The gross monetary returns was recorded the highest (Rs. 98730/-) in treatment of T_6 followed by treatment T_5 (Rs. 97349/-). Highest net return was recorded in treatment of T_6 (Rs. 49155/-). The benefit: cost ratio was found highest (2.17) in treatment of T_1, followed by T_6 (1.99) and T_5 (1.97).

5.2 Conclusion

It is concluded that, soil application of one week incubated zinc sulphate @ 20 kg ha^{-1} + 100 kg FYM or soil application of cow dung slurry with $ZnSO_4$ @ 20 kg ha^{-1} (1:4) @ 500 L ha^{-1} at 30 DAS through irrigation along with the general recommended dose of fertilizer (120:60:40 $N:P_2O_5:K_2O$ kg ha^{-1} + 10 t ha^{-1} FYM) to wheat was found beneficial for increase in grain and stover yield of wheat, total uptake of nitrogen, phosphorus, potassium, iron, zinc, manganese, copper, agronomic efficiency and zinc use efficiency, as well as for higher net monetary returns in wheat grown on zinc deficient medium deep black calcareous soil. The aforesaid treatments are being at par with treatment of GRDF+ seed coating treatment of chelated Zn EDTA @ 0.5% in respect of increase in zinc use efficiency, yield and monetary returns.

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7. VITAE

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IN

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