"EVALUATION OF MICRONUTRIENT FORMULATION IN COWPEA (*Vigna unguiculata* L. Walp)"

by

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(2017-11-144)

THESIS

Submitted in partial fulfilment of the

Requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY

COLLEGE OF AGRICULTURE

PADANNAKKAD, KASARGOD - 671 314

KERALA, INDIA

2019

DECLARATION

I, hereby declare that this thesis entitled **"Evaluation of micronutrient formulation in cowpea (*Vigna unguiculata* L. Walp)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate, fellowship or other similar title, of any other University or society.

Place: Padannakkad

Date : 30-8-2019

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CERTIFICATE

Certified that this thesis entitled **"Evaluation of micronutrient formulation in cowpea (*Vigna unguiculata* L. Walp)"** is a record of research work done independently by Ms. Roshni John under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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ACKNOWLEDGEMENT

First and foremost I bow my head before the Almighty who have given me the strength, courage and confidence to complete the thesis work successfully on time.

I extend my sincere gratitude and reverence to ***Dr. N. K. Binitha****, Assistant Professor, Department of Soil science and Agricultural Chemistry and the Chairman of my Advisory Committee for her advice, inspiration, unstinted attention and meticulous guidance and constant encouragement throughout the course of investigation. The keen interest, abundant encouragement, she has imparted to me throughout my research work are invaluable and because of which I have been able to successfully complete the work assigned to me.*

I wish to express my sincere gratitude to ***Dr. P. R. Suresh,*** *Associate Dean, Professor and Head, Department of Soil science and Agricultural Chemistry, College of Agriculture, Padannakkad and Member, Advisory Committee for his constant encouragement, support, technical guidance rendered at every stage of work and timely help throughout the research work and course of study.*

I express my heartfelt gratitude to ***Dr. T. K. Bridgit,*** *Professor and Head, Department of Agronomy, College of Agriculture, Padannakkad and Member, Advisory Committee for her timely suggestions and kind guidance throughout the course programme.*

I express my heartfelt gratitude to ***Sri. Retheesh P.K.,*** *Assistant Professor, Department of Agronomy for his valuable suggestions and guidance throughout the research work.*

I express my deepest and sincere thanks to ***Mr. Ajith Kumar****, Farm officer of RARS, Pilicode, all the staff members and workers for providing all necessary requirements for the conduct of research work.*

I take this opportunity to express sincere thanks to teachers ***Sri. Shameer, Dr. Nitheesh, Dr. Sudarsana Rao, Dr. Raji, Dr. Sreekumar, Dr. Susha, Dr. Sujatha, Dr. Rajesh*** *and I gratefully acknowledge the invaluable help I received* *from* ***Mr. Ashique, Ms. Beena*** *and* ***Ms. Amrutha, Ms. Ramya, Ms. Banu, Mr. Sukil and Mr. Manjunath*** *for the successfull completion of my research work.*

I wish to express my sincere gratitude to each and every teaching and non teaching staff of Agronomy, Horticulture, Agricultural Entomology, Plant Pathology, Agricultural Engineering and Agricultural Extension departments for their suggestions and guidance throughout the research work. I am thankful to my seniors, junior students and research assistants for their help during my research work.

I express my sincere thanks to my dear friends, ***Wayoo, Amalendu***, ***Anu, Giffy, Pathu, Glady, Jaseera, Reshma, Amrutha Radhika, Sonu, Suju, Ashni, Sajay, Chethan, Vinayak, Adarsh, Akhil*** *and* ***Shibin*** *my seniors* ***Ashwini, Mubarak***, ***Ebimol, Vineetha, Amrutha, Manju, Shaana, Laya and Sreelaja*** *and my juniors* ***Faizy*** *and* ***Nimya*** *for their constant help and encouragement that made me confident in difficult situations.*

I wish to acknowledge with gratitude the award of fellowship by the Kerala Agricultural University during the tenure of the M. Sc. (Ag.) programme.

I express my deep sense of gratitude and affection to my father ***Mr. John,*** *mother* ***Smt. Soosan,*** *brother* ***Ronald,*** *sister* ***Blessy*** *and my nephews* ***Jake, Jose, Varghese,*** *cousins and relatives for their affection, constant encouragement, moral support, prayers and blessings without which I would not have completed this research. Above all, for the attention focused and facilities arranged to carry forward my studies.*

Finally I thank all those people who have supported me during my post graduate programme and helped for the successful completion of this thesis.

Roshni John

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LIST OF ABBREVIATIONS

%	-	Per cent
@	-	at the rate of
B	-	Boron
°C	-	Degree celcius
Ca	-	Calcium
CD	-	Critical difference
CEC	-	Cation Exchange Capacity
cm	-	Centimeter
cmol kg^{-1}	-	Centimole per kilogram
COA	-	College of Agriculture
Cu	-	Copper
dS m^{-1}	-	deci Seimens per meter
DAS	-	Days after sowing
°E	-	East
EC	-	Electrical conductivity
et al	-	And others
Fe	-	Iron
Fig.	-	Figure
FYM	-	Farm Yard Manure
g	-	Gram
g cm^{-3}	-	Gram per centimeter cube
ha^{-1}	-	per hectare
hr^{-1}	-	per hour
K	-	Potassium
KAU	-	Kerala Agricultural University
kg	-	Kilogram

kg plant^{-1}	-	Kilogram per plant
kg ha^{-1}	-	Kilogram per hectare
l	-	Litre
m	-	Meter
meq 100g^{-1}	-	Milliequivalent per hundred gram
Mg	-	Magnesium
mg kg^{-1}	-	milligram per kilogram
ml	-	Milliliter
mm	-	Millimeter
Mn	-	Manganese
Mo	-	Molybdenum
MOP	-	Muriate of Potash
N	-	Nitrogen
^{0}N	-	North
NS	-	Not significant
O	-	Oxygen
OC	-	Organic carbon
P	-	Phosphorus
pH	-	Soil reaction
plant^{-1}	-	Per plant
POP	-	Package of practices
ppm	-	Parts per million
S	-	Sulphur
SE(m)	-	Standard error mean
viz.	-	Namely
Zn	-	Zinc

Introduction

1. INTRODUCTION

Pulses are an important group of crops which has the capacity to provide high quality protein complementing cereal proteins in vegetarian diet. The Recommended Dietary Allowances (RDA) for adult male and female is 60 g and 55g per day respectively. India being the largest pulse crop cultivating country in the world, the country's pulse production is relatively in comparison to total cereal production. Pulses can be cultivated with minimum resources and hence it is cheaper than animal protein sources. Though pulses are grown in both kharif and rabi seasons, rabi pulses contribute more than 60 per cent of the total production. In India, pulses are cultivated in an area of 25.26 million ha with production potential of 16.47 million tonnes and yield of 652 kg ha^{-1} (GOI, 2016). In Kerala it is cultivated in an area of 1738 ha with a production of 1433 tonnes (GOK, 2016-2017). Bengal gram, pigeon pea, green beans, black matpe, red kidney beans (rajma), black eyed peas (lobia), lentils (masoor), white peas (matar) are the major pulses grown and consumed in India.

Cowpea (*Vigna unguiculata* L. Walp) is an important pulse crop grown annually in warm climate with adequate rainfall. It can thrive well in temperate zones as well as humid tropics. Cowpea is rich in fibre, protein, iron and potassium but low in fat and calorie. It is also referred as vegetable meat because of its high protein content. Inclusion of cowpea in diet prevents cancer, anaemia and repair of muscle tissues. Cowpea is mostly grown under unirrigated condition and on marginal land, hence the yield is very low. Sufficient supply of plant nutrients according to crop needs increases the yield of crops. Yield potential of crops can be achieved by adequate supply of micronutrients along with major nutrients.

Micronutrients are essential elements in growth required by plants only in small quantities. In spite of its lower requirement in plants these are equally formidable as primary and secondary nutrients. Deficiency of micronutrients results in disrupted plant functioning and abnormal plant growth. It may further lead to yield reduction. Micronutrients are essential for the proper metabolism in animals also. Hence periodic monitoring of micronutrient status in soil must be carried out in order to supplement them in optimum levels. The deficiency of micronutrients become prevalent quite recently affecting the productivity and stability of soils (Bell and Dell, 2008). The factors which triggers micronutrient deficiency includes intensive cropping, leaching and loss of top soil, preference of macronutrient fertilizers over organic manure and inadequate management of soil (Singh, 2003; Rattan and Sharma, 2004; De and Rai, 2005).

Micronutrients can be supplied to plants as soil application, seed treatment and foliar application. Soil application of fertilizers leads to enormous wastage of the applied nutrients through leaching, run off and gaseous loss depending on the prevailing soil conditions for crop growth. This may lead to lowering the fertilizer use efficiency. Foliar nutrition is the most advanced method in supplementing nutrition to plants. It gains attention due to its efficiency as well as cost effectiveness. Application of micronutrients in the appropriate mineral form enhances its availability in plants (Alloway, 1986; House and Welch, 1989). Formulations containing micronutrients when supplied to plants as foliar spray are found effective in correcting deficiencies and thereby increases the yield (Mona *et al.*, 2012). Foliar application of nutrients helps the plants to absorb nutrients through the stomatal openings in leaves and results in most effective use of fertilizers in a most economical way (Manasa *et al.,* 2015). Efficiency of fertiliser use and quick response of the applied nutrients results in growth enhancement as well as yield. It also reduces the chance of toxicity in plants compared to the supplementation of fertilizers through conventional methods.

Seed treatment is the method of application of fungicides, insecticide or combination of both to prevent seed borne pathogens and insects. It also aimed at ensuring the quality of crops which in turn affects the germination, growth, development and yield of crops. Micronutrient fertilizers can also be supplied as seed treatment so that the wastage of fertilizers during soil application can be significantly reduced. Application of micronutrient through seed treatment improves growth, yield and enhances the phenological events (Farooq *et al.*, 2012). In economical perspective seed treatment is the better option for which less micronutrient is needed, it is ease in application and improves seedling growth (Singh *et al.*, 2003).

Biological nitrogen fixation accounts as a major source of nitrogen input in soils. Legume plants can fix atmospheric nitrogen by formation of nodules. Legume rhizobium symbiosis is an ideal solution to restore soil fertility (Zahran, 1999). Symbiotic association of legumes and nitrogen fixing rhizobium bacteria acts as a natural nitrogen factory of native soils. Biological nitrogen fixation is being widely exploited as it enhances the nutrient status of soil as well as increasing the growth and development of plants. Cowpea being a legume crop has the capacity to fix atmospheric nitrogen in symbiotic association with rhizobium species. Root nodules are formed in cowpea where these symbiotic bacteria are sheltered and thus nitrogen fixation occurs in areas where cowpea is cultivated. Since it restores soil fertility it is an important component of crop rotation. Cowpea is grown before cereals as it enhances the nitrogen status of the soil (Carsky *et al.*, 2002; Tarawali *et al.*, 2002; Sanginga *et al.*, 2003). All the nodules may not fix nitrogen efficiently. To enhance the nitrogen fixation capacity of nodules seeds are treated with rhizobium cultures of suitable strains according to crop species.

On this background the present investigation is proposed with the following objectives

- To evaluate the effect of micronutrient formulation through seed treatment and foliar nutrition on growth, nodulation and yield of cowpea
- To study its effect on plant nutrient uptake and residual soil nutrient status

Review of Literature

2. REVIEW OF LITERATURE

Nutrients are essential components which sustains plant as well as animal population. Plants need all 17 essential elements for its growth and development. It includes macronutrients and micronutrients. Micronutrients are referred as trace elements because plants need these nutrients in small quantities. Even though its requirement is less these nutrients are as important as macronutrients. Lack of any micronutrient element results in growth inhibition and results in complete death of plants (Mengel *et al.,* 2001). As it is involved in metabolic and other enzymatic activities their deficiency affects the growth of plants. Micronutrient status in the soil is decreasing nowadays due to the nonjudicious use of primary nutrient fertilizers. Inorder to maintain the soil nutrient status as well as nutrient requirement of the crops it is essential to supply micronutrients. The present investigation was carried to evaluate the effect of micronutrient application both as seed treatment and foliar application in growth, yield and nutrient uptake of cowpea.

2.1 IMPORTANCE OF COWPEA AS A LEGUME CROP

Cowpea (*Vigna unguiculata* L Walp) is an important vegetable crop belongs to the family Leguminosae. It is recognized as one of the major pulse crop in the world. It was domesticated primarily in Africa and later spread to different regions of the world including tropics and temperate climatic conditions (Pasquet, 1998). It grows well in tropical conditions as it can tolerate high temperature and withstand drought (Hall *et al.*, 2002; Hall, 2004). It can be grown as vegetable crop as well as for grain purpose.

In diet the nutritional quality of cowpea grain is almost same as common bean (Bressani, 1985). But folic acid and other antinutritional factors are very less in cowpea. Grain cowpea has gained importance in developing countries, facing acute shortage of fuels as grain cowpea requires less time for cooking (Hall and Ehlers, 1997).

Cowpea, a legume crop has the capacity to fix atmospheric nitrogen in symbiotic association with rhizobium species. Root nodules are formed in cowpea where the symbiotic bacteria are sheltered and fixes nitrogen in the legume cropped area. Since it restores soil fertility it is an important component of crop rotation. Cowpea is grown before cereals as it enhances the nitrogen status of the soil (Carsky *et al*., 2002; Tarawali *et al*., 2002; Sanginga *et al*., 2003). All the nodules may not fix nitrogen efficiently. To enhance the nitrogen fixation capacity of nodules seeds are treated with rhizobium cultures of suitable strains according to crop species

2.2 IMPORTANCE OF MICRONUTRIENTS IN AGRICULTURE

Essential elements which are required by plants in very low concentration are referred as micronutrients. Even though these nutrients are required in very low quantities they play a prominent role in the metabolism of plants (Benepal, 1967; Katyal, 2004). Micronutrients are essential for the functioning of various enzymes involved in plant system thus helping in nutrition and yield of crops (Kazi *et al*., 2012). The deficiency of these nutrients reduces the yield of crops tremendously (Udode- Haes *et al*., 2012). High nutrient requirement of modern crop cultivars aggravates deficiency of micronutrients (Fageria *et al*., 2002).

All micronutrients except boron and chlorine are metals and its uptake is influenced by several factors including environmental and microbial (Romheld and Marschner, 1986; Clark and Zeto, 2000), plant (Barber, 1995; Marschner, 1995) and soil (Lindsay, 1991; Lake *et al*., 1984).

2.2.1 Iron

Iron is an essential micronutrient element needed for the growth of plants (Gris, 1843). It is a constituent of various enzymes including cytochrome oxidase, catalase and nitrogenase. It also helps in respiration, photosynthesis and reduction of nitrates and sulphates (Wallihan *et al*., 1958; Reddy and Reddi, 2002).

Iron is an important constituent of leghaemoglobin in legumes (Gupta and Gupta, 2005). Leaf chlorosis due to deficiency of iron can be reduced by the application of iron fertilizers (Srivastava and Singh, 2003).

2.2.3 Manganese

Essentiality of manganese for plants was proposed by Mchargue (1922). It is essential for photosynthesis, respiration and activity of enzymes. Mn is an important component in Krebs cycle (Gupta and Gupta, 2005).

Manganese deficiency is observed in younger leaves resulting in light green mottles between the veins (Gupta and Gupta, 2005). Foliar spray of manganese sulphate at 0.1 per cent is recommended (Gupta and Gupta, 2005) for manganese deficiency.

2.2.4 Zinc

Sommer and Lipman in 1926 discovered the essential micronutrient zinc. It influences the growth and development of plants. Zinc is a limiting micronutrient in crop production. It is a constituent of enzymes which produces proteins and helps to retain the bonds of biomembranes. It is involved in the synthesis of indole acetic acid. It is essential for metabolism of carbohydrates, synthesis of proteins, internode elongation and in pollen formation (Shukla *et al.*, 2009).

Zinc deficiency results in growth retardation and little leaf (Marschner, 1995). Thus reducing the yield and quality of crops (Gupta, 1995). Zinc spray on necrotic leaves found to increase the green pigments (Srivastava and Singh, 2003).

2.2.5 Copper

Essentiality of copper as micronutrient was put forward by Lipman and MacKinney (1931). It is essential for photosynthesis and mitochondrial respiration. In chloroplasts the electron carrier proteins such as plastoquinone and plastocyanin contain Cu (Gupta and Gupta, 2005).

2.2.6 Boron

Essentiality of boron was first discovered by Warrington (1923). The major roles of boron in plants include promoting plant growth, meristem cell division, translocation of sugars, starch, nitrogen, phosphorus, development of phloem and help in calcium metabolism. Lack of boron in cells results in breakdown of phloem tubes (Edmond *et al.*, 1997).

2.2.7 Molybdenum

Essentiality of molybdenum for plants was identified by Arnon and Stout (1939). It is a component of nitrate reductase and nitrogenase. Also of enzyme oxidase which results in the conversion of abscisic acid aldehyde to ABA (Gupta and Gupta, 2005).

2.3 MICRONUTRIENTS STATUS IN INDIAN SOIL

According to Katyal and Sharma (1991) higher concentrations of micronutrients such as Fe, Mn, Zn, Cu were found in soils developed from green schist and these were too low in sandstone. Limestone, a sedimentary rock contains highest concentration of Zinc.

Soils formed on flood plain alluvium found to be lower in Zn, Cu, Mn and Fe content. Sedimentary rocks contain more Boron compared to igneous rocks (Gupta and Gupta, 1985).

Micronutrients present in the lithosphere are essential for survival of plants. The least abundant micronutrient element in lithosphere is Molybdenum (Mortvedt, 2000). Molybdenum is available to plants only at alkaline pH and it is deficient in acid soils. Poorly drained soil and peaty soils have high molybdenum content.

Unavailability of micronutrients limits growth of plants. Fe is an important micronutrient whose deficiency limits plant growth. Availability of iron to plants is mostly affected with different cropping sequences because of frequent changes in the form of iron ie, from Fe^{2+} to Fe^{3+}.

Boron is an important micronutrient element which ranges at the rate of 7 to 630 mg kg^{-1} in Indian soils (Prasad *et al.*, 2014). Total Zn content in Indian soils ranges from 7 to 284 ppm in entisols and vertisols respectively (Ganjir *et al.*, 1973).

2.4 MICRONUTRIENT DEFICIENCIES IN INDIAN SOILS

The advent of green revolution in India during 1960s increased the crop production in our country (Singh, 2009). Application of chemical fertilizers in fields promote higher production. The injudicious use of macronutrient fertilizers resulted in micronutrient deficiency in Indian soils. Deficiencies of nutrients including both macro and micronutrients are prevalent in Indian soils (Takkar *et al.,* 1989).

The antagonistic interaction of zinc and phosphorus is quite evident that is, soils with higher amount of phosphorus reduces the uptake of zinc from soil (Dadhich and Somani, 2007; Kizilgoz and Sakin, 2010).

Zinc is deficient in soils containing low organic matter and high pH (Rattan and Sharma, 2004). Zinc associated with solid particles cannot be taken by plants (Lake *et al.*, 1984).

Boron is an important micronutrient which is deficient in Indian soils (Sathya *et al.*, 2009). According to Katyal and Vlek (1985), B deficiency in Indian soils was initially reported as 2 per cent during 1980 and it reached to 53 per cent in 2012 (Singh, 2012). B is deficient in Indian soils with a mean of 33 per cent in the whole country (Singh, 1999; Singh, 2006).

Shukla *et al.* (2012) reported that wheat grown after rice cultivation was found to be deficient in manganese. It was observed mainly in wheat grown tracts of Punjab and Haryana. The continuous leaching of Mn was found to be the reason for Mn deficiency. Deficiency of micronutrients affects the productivity of crops which in turn affect the health of man and animals.

Plant samples were collected to study the micronutrient concentration (Shukla *et al.*, 2012). About 20,000 plants were taken for the analysis of Fe, Mn, Zn and Cu concentrations. Study showed that plants were deficient in Fe by 6 per cent, Mn by 4 per cent, Zn by 44 per cent and Cu by 10 per cent

Iron is deficient in most soils. Deficiency in soils are reflected in plants and animals as deficiency symptoms and disorders. This condition can be eliminated by increasing the levels of available iron in staple food crops (Mori, 1999).

2.5 MICRONUTRIENT STATUS IN KERALA SOILS

A major portion of Kerala soils lack boron since it is formed from acid igneous rocks (SSO, 2007). Highly soluble nature of boron in soil (Tisdale *et al.*, 1985) is one of the reason for its deficiency in Kerala, which receives a major share of rainfall during monsoon. A study conducted by Kavitha and Sujatha, 2015 in various agroecosystems of banana, coconut, nutmeg, paddy, rubber, vegetables and pepper in Thrissur district showed that all the micronutrient elements except boron are in sufficient range.

A study conducted by Suresh *et al.*, 2014 in the Northern district of Kerala, Kasaragod revealed that the soils are deficient in boron by 78 per cent, zinc by 8 and copper by 3 percentage.

Acid sulphate soils in the Kuttanad region of Kerala is found to be toxic with Fe and Al which reduces the crop yield (Thampatti *et al.*, 2005).

2.6 ROLE OF RHIZOBIUM IN GROWTH, YIELD, NODULATION AND NUTRIENT UPTAKE OF CROPS

Co-inoculation of plant growth promoting rhizobacteria and rhizobium in the soil contaminated with Cu was studied in alfalfa. The results suggested that the combination increased the growth of plants as well as Cu uptake by plants. Thus it eliminates Cu stress and improves biochemical properties of soil (Ju *et al.*, 2019).

Efficient fixation of nitrogen occurred when the legume host and rhizobium were compatible to each other. This compatibility is not only essential for infection but also for proper development and nutrient exchange mechanisms (Terpolilli *et al.*, 2012).

Seeds of chickpea were inoculated with rhizobium culture alone and in combination with N at the rate of 25 kg N per ha. The study revealed that there was significant increase in nodulation of chickpea compared to control. Increase in yield was also prominent (Tilak *et al.*, 1981).

According to Nyoki and Ndakidemi (2018), when soybean seeds were inoculated with rhizobium the uptake of macronutrients such as N, P, K and Mg was significantly increased in soybean shoots. This study also revealed that the uptake of micronutrients such as Fe, Mn, Zn, Cu increased prominently.

Wolde-meskel *et al.* (2018) studied the effect of rhizobium inoculation and phosphorus application in chickpea. Inoculation and application of P increased the grain yield of chickpea by 21 per cent and 25 per cent respectively, while the combination of both treatments increased the yield by 38 per cent.

Effect of rhizobium inoculation along with the application of N and P fertilizers was studied in summer forage cowpea. The results showed that the application of nitrogen and phosphorus fertilizers at the rate of 20 kg ha^{-1} and 40 kg ha^{-1} respectively along with rhizobium inoculation increased the yield and profit of summer cowpea (Amrutrao, 2018).

Inoculation of rhizobium culture in seeds of cowpea do not show significant effect on seed and fodder yield but it showed significant effect on the nitrogen content of seeds as well as P, K and Mg content of fodder (Reddy, 1981).

Plant height, number of branches and yield of garden pea were increased in seeds treated with rhizobium culture (Patel, 1994).

Number of nodules and dry weight of nodules were found to be more in cowpea plants treated with vermicompost along with rhizobial inoculation compared to FYM + rhizobial inoculation (Yadav, 2000).

Mathur (2000) reported that in mungbean inoculation of rhizobium significantly increased growth parameters. Growth parameters include nodule formation, plant height and nodule number.

Plant height, LAI, number of nodules and branches per plant were recorded more in plants inoculated with rhizobium (Thakur *et al.*, 1999). Cowpea seeds inoculated with rhizobium showed higher dry matter production compared to uninoculted seeds (Yadav and Malik, 2005).

The rate of dry matter production in cowpea was observed to increase from 39.1 to 44.5 kg ha^{-1} per day compared to uninoculated plots with dry matter production ranges from 35.4 to 37.4 kg ha^{-1} per day (Deshmukh and Joshi, 1973).

Yield response of cowpea treated with rhizobium culture was studied by Lehri *et al.* (1974). It was observed that the yield of cowpea increased from 17.2 to 28.7 q ha^{-1}.

When seeds of *Phaseolus aureus* were treated with rhizobium bacteria, there occurred a significant increase in shoot and root length, fresh and dry weight of plants (Madani and Singh, 1977).

In temperate conditions cowpea seeds inoculated with rhizobium showed increased number of leaves, weight and nodulation compared to the control (Pant *et al.*, 2000).

2.7 SIGNIFICANCE OF NITROGEN FIXATION IN SOIL FERTILITY

Fixation of nitrogen in soil with the help of nitrogen fixing microorganisms is referred as biological nitrogen fixation. It plays a prominent role in the establishment of crop and increase in yield. It provides nitrogen needed for the growth and development of plants even when the crop is deprived of N fertilizers (Vargas and Hungria, 1997; Chen *et al.*, 2002).

Methods to replenish the nitrogen status of the soil are application of nitrogenous fertilizers, organic manures and growing legume crops which fix atmospheric nitrogen in symbiotic relation with rhizobium bacteria referred as Biological Nitrogen Fixation (BNF). But the rate of BNF is declining nowadays due to unjudicious use of nitrogenous fertilizers (Peoples *et al.*, 1995).

Soil erosion is one of the major reason for soil degradation which results in removal of top soil and leave the soil as infertile. Biological nitrogen fixation plays an important role in replenishing the nitrogen status of such soils (Giller and Cadisch, 1995).

In Brazil cereals were grown with N supply much less than the required quantity along with the association of diazotrophic bacteria. P, K and other micronutrients were supplied in ample quantities. It was observed that the crop was able to obtain up to 30 per cent of their required nitrogen from biological nitrogen fixation (Dobereiner, 1997).

The amount of nitrogen produced in the soil by decomposition of roots and nodules of legumes has been estimated as 3 to 120 kg N $ha^{-1}yr^{-1}$ or 2 to 26 per cent of biological nitrogen fixation (Ledgard and Steele, 1992). Thus improves the nitrogen status of soil.

2.8 SIGNIFICANCE OF MICRONUTRIENT SEED TREATMENT IN CROPS

Micronutrient seed treatment is equally beneficial compared to other methods of micronutrient application in crops. (Farooq *et al.*, 2012).

Pulse crops treated with Mo in the form of sodium molybdate at the rate of 10g per 25 kg of seed increased the seed yield by 45 per cent compared to non molybdenum treated seeds (Pranakrishna, 1976).

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Cowpea seeds were treated with micronutrients such as zinc and boron to study its effect on growth and yield. Seeds treated with borax showed greatest plant height at 30 and 60 DAS. Seeds treated with zinc sulphate showed highest root length (18.51 cm), vigour index (4277) and seedling dry weight (0.595 g) (Masuthi *et al.*, 2009).

Common bean treated with zinc increased the yield and other traits (Kaya *et al.,* 2007). Seeds treated with micronutrients showed improved uptake of zinc and phosphorus in barley. Accumulation of dry matter was also found to be increased (Ajouri *et al.,* 2004).

Wheat seeds treated with 0.3 % zinc increased the yield by 14 per cent and also chickpea seeds treated with 0.05 % zinc increased the yield by 19 per cent (Harris *et al.,* 2008).

In kidney beans seed treatment with zinc alone did not meet the requirement of the crop (Rasmussen and Boawn, 1969). This condition depends on the amount of zinc present in the soil. In case of moderate deficiency seed treatment with zinc is economic (Harris *et al.,* 2007, 2008; Slaton *et al.,* 2001) but not suitable under severe deficiencies (Rasmussen and Boawn, 1969; Rehman *et al.,* 2012).

Rice seeds treated with 0.001 per cent and 0.1 per cent boron solutions improved the crop establishment while 0.5 per cent boron solution failed to germinate (Rehman *et al.,* 2012).

Papaya seeds treated with 2 ppm boron solution for 6 hours showed increased germination and better growth of seedlings (Deb *et al.,* 2010). Plant height, fruiting and pod yield were higher in pea seeds treated with 0.5 per cent boron solution (Kumar *et al.,* 2008).

Seed treatment with boron did not influence the yield of chickpea, lentil, rice or wheat but it increased the boron content of grains in all these crops (Johnson *et al.,* 2005).

Seed treatment is the most efficient method for the supply of molybdenum for plants compared to soil application (Donald and Spencer, 1951; Johansen *et al.,* 2006).

According to Kumar *et al.* (2004) chickpea seeds treated with molybdenum 0.5 gram per liter solution of sodium molybdite for 8 hours resulted in increase in yield by 27 per cent compared to soil application.

Seed treatment of green gram seeds with sodium molybdate along with rhizobium culture increased nodulation, nitrogen fixation, nutrient uptake, plant growth and yield (Pattanayak *et al.,* 2000).

Wheat seeds treated with manganese sulphate solutions showed improved growth, grain yield and grain manganese contents (Khalid and Malik, 1982). Seeds treated with Mn solution showed better growth and yield in *Echinacea purpurea* (L.) compared to soil application (Babaeva *et al.,* 1999).

Wheat seeds treated with CuEDTA solution increased the grain yield but seedling emergence was suppressed (Malhi, 2009). Oat seeds treated with 0.001 per cent copper sulphate solution did not show significant effect on germination but number of grains per panicle and grain weight increased resulting in 16.53 per cent increase in yield compared to untreated samples (Saric and Saciragic, 1969).

2.9 FOLIAR APPLICATION OF MICRONUTRIENTS

Application of micronutrients as foliar spray is effective in controlling deficiencies of crops because it gives immediate results compared to soil application (Torun *et al.*, 2001; Jamal *et al.*, 2006). The immediate action of foliar sprays is due to faster absorption of nutrients through leaf surface than through roots (Hashemy *et al.*, 1998).

Yield and quality of crops can be enhanced through foliar nutrition (Roemheld and El-Fouly, 1999; Sarkar *et al.*, 2007). Foliar nutrition is a better alternative in arid and semiarid regions compared to soil application (Kaya *et al.*, 2005; Kinaci and Gulmezoglu, 2007; Babaeian *et al.*, 2011).

Fertilizer use efficiency can be increased by foliar nutrition as it requires nutrients in smaller concentrations and thus prevents toxicity and fixation (Malakouti and Tehrani, 1999; Silberbush, 2002).

Foliar feeding is the most accepted method of plant nutrition in modern world (Bernal *et al.*, 2007 and Baloch *et al.*, 2008). It is cost efficient because of its less requirement compared to soil application (Kannan, 1986 and Singh, 2007). It started during 1950 s in America and later adopted by many countries (Wiley-VCH and Wiley-VCH, 2007).

The mechanism of entry of nutrients through leaves is a step by process. It has to penetrate the leaf surface through cuticle, stomata, leaf hairs and other epidermal cells (Fernandez and Eichert, 2009).

Leaf can quickly absorb the nutrients supplied through foliar spray and transport it to phloem and xylem vessels (Hasslett *et al.*, 2001; Nasiri *et al.*, 2010). It also increases the uptake of nutrients through soil by promoting root growth (El-Fouly and El-Sayed, 1997). Thus foliar nutrition is important nowadays compared to soil application (Alam *et al.*, 2010).

Foliar feeding is important in plant metabolism as it increases photosynthetic pigments and organic components in *Cassia angustifolia* (Shitole and Dhumal, 2012).

Foliar feeding is more advicable than soil application as it is 6 to 20 times more efficient (Liew, 1988). Zn, B and Cu foliar spray is equally benefited as soil application in reducing sub soil micronutrient deficiency (Ali *et al.*, 2009; Hussanin *et al.*, 2012).

Grain yield and protein content in wheat, maize, rice, barley and sorghum was enhanced by foliar nutrition of trace elements (Boorboori *et al.*, 2012). Shoot concentration and uptake of nutrients was found to be more in soybean plants treated with Fe and Mn foliar spray (Moosavi and Ronaghi, 2011).

Thus foliar nutrition is an effective method of supplying essential nutrients for the growth of plants.

2.10 MICRONUTRIENT APPLICATION IN GROWTH, YIELD AND NUTRIENT UPTAKE OF CROPS

Micronutrients enhances the growth and yield of crops (Simmonds, 1966). A micronutrient mixture containing Zn, B, Cu, Mg and N at 9.5, 2.6, 1.2, 2.4 and 0.46 per cent respectively was developed. Application of this mixture at 0.5 per cent in bhindi variety Varsha Uphar increased the growth and yield of crops (Mini, 2015).

El-Magid *et al.* (2000) reported that application of nitrogen, phosphorus and potassium along with foliar spray of micronutrient mixture (Fe, Mn, Cu, Zn, B, Mg, S and Mo) enhanced yield of rice.

Application of 0.1 per cent micronutrient mixture containing B, Fe, Cu, Co, Zn and Mn increased the grain yield of rice and reduced spikelet sterility (Shueadshen, 1991).

Foliar application of humic acid and zinc sulphate at 20 ppm and 25 ppm respectively produced higher yield in paddy (Durairaj, 1993). In cotton highest yield was recorded by the application of magnesium sulphate at 1 % and zinc sulphate at 0.5 % during squaring, flowering and boll formation (Katkar, 2005).

Yield of okra is increased by the foliar application of multi-micronutrient mixture (Patel *et al.*, 2009). Micronutrient mixture containing Zn, Cu, B and Fe chelated with organic components sprayed at 2 per cent increased height, leaf area and yield of okra (Datire *et al.*, 2010).

Foliar application of Agromin (Fe chelated with Zn, Cu, Mn, Mg, B and Mo) increased the growth of okra (Suryanarayana and Rao, 1981). In pumpkin fruit and seed yield was found to be increased by the foliar spray of zinc and manganese (Yousefi and Zandi, 2012).

Mishra *et al.* (2012) studied the effect of foliar spray of micronutrient mixture containing Zn, B, Fe, Mn, Cu at 100 ppm each and Mo at 50 ppm in tomato. He reported increase in number of fruits per plant and thus enhancing the yield.

Sivaiah *et al.* (2013) reported that combination spray of micronutrients or single micronutrient spray increases the plant height, fruit weight, number of branches and seed yield in tomato.

Selvi and Thiageshwari (2002) reported that micronutrient leaf nutrition increased the fruit yield of bhindi. Foliar nutrition of zinc sulphate at 0.5 % and borax at 0.2 % produced highest shoot length, number of internodes and more number of leaves per shoot of grapes (Singaram and Prabu, 2001).

Micronutrient mixture containing zinc sulphate + iron sulphate + Borax sprayed over pomegranate and found out an increase in fruits per plant and fruit weight (Afria *et al.*, 1999). Leaf length, number of leaves and floret of gladiolus was increased by the foliar spray of zinc at 0.5 per cent (Katiyar *et al.*, 2012).

Application of boron as foliar spray increased the plant height, leaf number and root weight in tomato (Verma *et al.,* 1973). Tomato cv. Dhanashree recorded highest number of fruits when 50 ppm boron was sprayed (Mishra *et al.*, 1990).

Foliar spray of boron and zinc each at 2 % and 6 % respectively increased plant height by 30 per cent compared to the control in tomato (Ejaz *et al.*, 2011). Foliar application of 1 % iron sulphate in onion showed increase in yield and plant height (Mishra *et al.*, 1990).

Plant height and root length of green gram plants treated with Fe was found to be increased (Prakash, 1998). Application of sulphur along with zinc and iron increased the growth, yield and nutrient uptake of safflower (Ravi *et al.*, 2008).

Foliar spray of 1 per cent micronutrient mixture at 30, 40 and 50 DAS increased plant height, tiller number and nutrient uptake in rice. The micronutrient mixture was comprised of 4 % Fe, 1 % Mn, 6 % Zn, 0.5 % Cu and 0.5 % B (Patel *et al.,* 2008).

Foliar spray of borax at 0.5 % in potato at 35 days after planting increased the nutrient concentration in tuber and also increased the nutrient uptake of crops (Das, 1977). Application of micronutrients such as Fe, Mn, Zn, B, Mo and Cu increased the uptake of N,P,K,Ca and Mg in soybean (Thiyageshwari and Ramanathan, 2001).

Groundnut plants treated with molybdenum at the rate of 4 g kg^{-1} seed increased the yield of pod by 131.9 per cent compared to the control (Wankhade *et al.*, 1992). Battacharya *et al.* (1997) showed that the combined application of Mo at 3.0 kg/ha with S and Zn increased the dry weight of groundnut plants by 117 %.

Khanal *et al.* (2005) and Bhagiya *et al.* (2005) studied the effect of molybdenum in chickpeas, mungbean and groundnuts. They recorded a significant increase in pod yield and seed yield.

Gad (2012) recorded that application of molybdenum significantly increased the minerals composition such as N, P, K, Fe, Mn, Zn, Cu and Mo in groundnut seeds under different levels of nitrogen (25, 50, 75 and 100 %) compared with untreated seeds.

Groundnut treated with Mo at the rate of 2 to 4 g per kg seeds showed increased number of nodules and dry weight per plant (Wankhade *et al.,* 1992). Combined spray of micronutrients such as cobalt, boron and molybdenum enhanced the uptake of N, P and S in pea (Singh *et al.*, 2012). It also increased the number of nodules and weight of nodules.

2.11 MICRONUTRIENT APPLICATION IN COWPEA YIELD, NODULATION, NUTRIENT UPTAKE

Foliar application of boron in cowpea showed significant increase in number of pods per plant and pod yield per plant which was recorded as 26 per cent and 13 per cent respectively and was found to be higher over the control (Chatterjee and Bandyopadhyay, 2017).

Foliar spray of 0.5 per cent $FeSO_4$ and 0.5 per cent $ZnSO_4$ at 45 days after sowing show increased seed yield in cowpea plants by 43.09 per cent compared to the control. It is then followed by combination spray of 0.5 per cent $FeSO_4$ and 0.5 per cent $ZnSO_4$ at 25 days after sowing (40.14 %). Benefit cost ratio also followed the same (Anitha *et al.*, 2005).

Foliar spray of three different concentrations (0, 1 and 2 ppm) of micronutrients such as Fe, B and Zn were sprayed at an interval of every 15 days in cowpea. Nutrient concentration and seed protein content was found to be highest in 1 ppm spray. Foliar spray with iron showed greater nutrient uptake and protein percentage in seeds compared to others (Salih, 2013).

In cowpea maximum nodulation was obtained in soils applied with 5 kg ha^{-1} and 20 kg ha^{-1} boron and manganese respectively (Rhoden and Allen, 1982). Cowpea plants supplied with Mo as spray recorded increased pod dry weight by 271.7 per cent compared to the control. Same trend was observed in soybean which showed an increase in pod weight by 258.3 per cent (Vargas and Ramirez, 1989).

Niranjana *et al.* (2005) stated that molybdenum at 2.0 g kg^{-1} seed recorded the highest growth parameters and pods yield in cowpea compared the control. Molybdenum application in cowpea plants resulted in increased number of nodules and dry weight. It also enhanced the rate of nitrogen fixation (Krishnappa *et al.*, 1992 and Noor *et al.,* 1997). Application of molybdenum on cowpea plants results in enhanced accumulation of nutrients in plant tissues (Hristozkova *et al.,* 2006).

Francois (1989) studied the effect of boron on growth of crops. He observed that the relative yield of *P. vulgaris* decreased by 12.1 per cent and seed yield of cowpea was decreased by 11.5 % with each unit increase of boron in soil solution above 1 and 2.5 mg B per litre, respectively.

Application of $ZnSO_4$ at the rate of 25 kg per ha through soil increased the seed yield of cowpea by 43 per cent followed by foliar spray of 0.5 per cent Zn at 25 and 45 days after sowing. Number of pods per plant and number of branches were increased resulting in yield enhancement (Patel *et al*., 2011).

Cowpea seeds treated with *Bradyrhizobium japonicum* showed significant increase in the uptake of micronutrients by shoot (Zn = 47.6 %, Cu = 46.8 %, Fe = 24.3 % and Mn = 56.3 %), root (uptake of Zn, Cu, Fe and Mn by 54.9 %, 26.3 %, 63.7 %, and 34.8 % respectively) and pod (Zn = 36.5 %, Cu = 16.1 %, Fe = 72.5 % and Mn = 26.3 %) (Nyoki and Ndakidem, 2014).

According to Vieira *et al* (1998) application of molybdenum restricts the nodules senescence and thus maintaining a longer period of effective N_2 fixation. Foliar spray of molybdenum (0 and 40 kg ha^{-1}) and nitrogen as side dressing increased the total shoot nitrogen in common bean.

2.12 MICRONUTRIENTS IN PEST AND DISEASE CONTROL

Seeds treated with micronutrient showed resistance to soil borne pathogens in soybean. Higher the zinc content in seeds resist diseases during germination and development of seedlings (Marschner, 1995).

Essential nutrients influence the severity of diseases in plants (Huber and Graham, 1999). Optimum level of nutrients availability to plants is a better way to reduce the incidence of pest and disease in an integrated system of pest management (Graham and Webb, 1991).

Fe, Mn, Zn, Cu and B are deficient in most places of India. This results in deficiency disorders in plants. Application of nutrients to correct these disorders helps in developing resistance to diseases (Agrios, 2005).

Providing sufficient nutrients for plants reduces incidence of pest and diseases (Huber and Wilhelm, 1988). Sufficient amount of boron in plants reduces the chance of infestation while deficiency make plants susceptible (Gupta, 1993; Graham and Webb, 1991).

Manganese content reduces disease incidence. Micronutrients affects the severity of pathogens in host (Engelhard, 1990; Graham and Webb, 1991; Huber, 1980). Deficiency of micronutrients enhances disease severity (Engelhard, 1990; Graham and Webb, 1991; Huber, 1980; Fageria *et al.,* 1997; Baligar *et al.*, 1998).

Foliar spray of micronutrient solution on cucumber is a prophylactic method to avoid powdery mildew development (Reuveni *et al.*, 1998). Manganese fertilization in wheat reduced the incidence of powdery mildew and take-all (Simoglou and Dordas, 2006). Soil application of zinc reduced root rot in wheat (Grewal *et al.*, 1996).

Iron nutrition increases the vigour of plants and also inhibits the growth of pathogens in the rhizosphere. Iron nutrition reduced the severity of rust and smut in wheat (Graham and Webb, 1991; Graham, 1983).

Downey mildew of grapes can be controlled by soil application of Cu fungicides (Evans *et al.*, 2007). Ascochyta blight in beans and peas can be controlled by the nutrition of Mo in plants (Patil, 1981). In cucumber powdery mildew can be controlled by foliar application of borax, copper sulphate and manganese chloride (Reuveni *et al.*, 1998).

Materials and Methods

3. MATERIALS AND METHODS

An investigation entitled "Evaluation of micronutrient formulation in cowpea (*Vigna unguiculata* L. Walp)" was carried out at College of Agriculture, Padannakkad and Regional Agricultural Research Station (RARS) farm, Pilicode during 2017 to 2019. The objectives of the study were to evaluate the effect of micronutrient formulation through seed treatment and foliar nutrition on growth, nodulation and yield of cowpea (*Vigna unguiculata* L. Walp) and to study its effect on plant nutrient uptake and residual soil nutrient status. The study was conducted in three parts.

Part I: Standardization of micronutrient formulation for cowpea

Part II: Studies on time and dose of micronutrient formulation for seed treatment in cowpea

Part III: Field experiment to evaluate the micronutrient formulation in cowpea

3.1 STANDARDIZATION OF MICRONUTRIENT FORMULATION FOR COWPEA

Micronutrient formulation was standardized by Smt. Premalatha (2016), COA Padannakkad in tissue culture banana as part of her MSc. thesis work. It was then modified and standardized for bhindi by Ashwini (2018), MSc., COA, Padannakkad. Micronutrient formulation is a mixture of two solutions *viz.* solution A and solution B. Solution A is a mixture of zinc sulphate, boric acid, copper sulphate, manganous sulphate, ferrous sulphate and ammonium molybdate whereas solution B is an organic acid which act as a chelate. These two solutions have to be mixed in a specific ratio at the time of application because of the poor shelf life of micronutrient mixture. Attempt was being made to increase the shelf life of this micronutrient formulation in the combined form of both solutions A and B. Several trials were done and a combination of Solution A and Solution B were prepared so that it can be supplied to farmers as micronutrient mixture which reduces the difficulty of mixing solutions during field application. The micronutrient formulation can be diluted to different concentrations for seed treatment and foliar spray.

3.2 SEED TREATMENT STUDY

A laboratory study was carried out at COA, Padannakkad with different concentrations of micronutrient formulation to standardize the time and dose for seed treatment. The experiment was carried out in completely randomized design with seven treatments and three replications. Seeds treated with different concentrations of micronutrient formulation were kept in petri plates for germination studies which was then planted in pots. Each replication contained four plants and the total number of plants were 84. Soil, sand and cowdung were mixed uniformly in the ratio 1:1:1 to prepare the potting mixture. Plants were grown in the pots and observations were recorded upto three leaf stage.

3.2.1 Experimental details

Crop : Cowpea

Variety : PGCP 6 (Pant Lobia 3)

Spacing : 30 cm x 15 cm

Design : CRD

Treatments : 7

Replications : 3

3.2.2 Treatment concentrations

T_1 : Micronutrient formulation at the rate of 0.25 %

T_2 : Micronutrient formulation at the rate of 0.50 %

T_3 : Micronutrient formulation at the rate of 0.75 %

T_4 : Micronutrient formulation at the rate of 1 %

T_5 : Micronutrient formulation at the rate of 1.5 %

T_6 : Micronutrient formulation at the rate of 2 %

T_7 : Control (Seed treatment with water)

3.2.4 Biometric observations

The biometric observations included germination percentage, days taken for germination, seedling length at three leaf stage and seedling vigour index were recorded at 15 days after planting.

3.2.4.1 Germination percentage (%)

Germination percentage of cowpea seeds was noted by keeping it in moist filter paper.

3.2.4.2 Days taken for germination

Days taken for germination were recorded by counting the number of days taken for whole seeds to germinate.

3.2.4.3 Seedling length at three leaf stage (cm)

Plant height was measured from base of the plant to tip at two weeks after planting.

3.2.4.4 Seedling vigour index

Seedling vigour index was calculated by multiplying germination percentage with seedling length.

3.3 FIELD EXPERIMENT

The field experiment was carried out at Regional Agricultural Research Station (RARS), Pilicode to evaluate the effect of micronutrient formulation in cowpea. The experiment was conducted in randomized block design with 12 treatments replicated three times. The treatment consisting of combination of four levels of seed treatment (no seed treatment, seed treatment with rhizobium, seed treatment with best concentration from experiment 1 and second best seed treatment from experiment 1) and three levels of foliar application of micronutrient (no foliar application, one foliar application at 15 DAS and two foliar application at 15 and 30 DAS). Manures and fertilizers application and other cultural practices were followed as per POP, KAU (2016) for all the treatments uniformly.

3.3.1 Location and climate of area

Geographically, the experimental site was located at 12° 12' N latitude and 75° 10' E longitude and at an altitude of 15 m above mean sea level. The region mostly experiences a warm humid tropical climate.

3.3.2 Experimental site

The soil type of experimental field was red loam. Land is mainly used for cultivation of vegetables and tuber crops. Cassava was cultivated in the plot before the commencement of experiment.

3.3.3 Design and layout

The layout of the field is given in Plate 1. The details of experiment are given below.

Crop	: Cowpea
Variety	: PGCP 6 (Pant Lobia 3)
Spacing	: 30 cm x 15 cm
Plot size	: 3 m^2
Design	: Factorial RBD
Treatments	: 12
Replications	: 3
Date of sowing	: 20^{th} October 2018
Date of first harvest	: 24^{th} December 2018

EXPERIMENTAL LAYOUT

N

R_1T_7	R_2T_{12}	R_3T_2
R_1T_4	R_2T_5	R_3T_9
R_1T_1	$R_2T_{10}2$	R_3T_3
R_1T_5	R_2T_6	R_3T_{11}
R_1T_{10}	R_2T_2	R_3T_6
R_1T_{12}	R_2T_7	R_3T_4
R_1T_8	R_2T_9	R_3T_1
R_1T_6	R_2T_{11}	R_3T_{10}
R_1T_{11}	R_2T_3	R_3T_7
R_1T_3	R_2T_8	R_3T_{12}
R_1T_9	R_2T_4	R_3T_8
R_1T_2	R_2T_1	R_3T_5

Plate: 1 – Layout of the field experiment

3.3.4 Treatments

Factor A : Seed treatment

S_1 : No seed treatment

S_2 : Seed treatment with rhizobium

S_3 : Seed treatment with micronutrient formulation (best seed treatment from experiment 1)

S_4 : Seed treatment with micronutrient formulation (second best seed treatment from experiment 1)

Factor B : Foliar application

F_1 : No foliar application

F_2 : One foliar application at 15 DAS

F_3 : Two foliar application at 15 and 30 DAS

Treatment details

T_1 : No seed treatment and no foliar application (S_1F_1)

T_2 : No seed treatment + one foliar application (S_1F_2)

T_3 : No seed treatment + two foliar application (S_1F_3)

T_4 : Seed treatment with rhizobium + no foliar application (S_2F_1)

T_5 : Seed treatment with rhizobium + one foliar application (S_2F_2)

T_6 : Seed treatment with rhizobium + two foliar application (S_2F_3)

T_7 : Seed treatment with best treatment from experiment 1 + no foliar application (S_3F_1)

T_8 : Seed treatment with best treatment from experiment 1 + one foliar application (S_3F_2)

T_9 : Seed treatment with best treatment from experiment 1 + two foliar application (S_3F_3)

T_{10} : Seed treatment with second best treatment from experiment 1+ no foliar application (S_4F_1)

T_{11} : Seed treatment with second best treatment from experiment 1+ one foliar application (S_4F_2)

T_{12} : Seed treatment with second best treatment from experiment 1+ two foliar application (S_4F_3)

3.3.5 Crop cultivation details

3.3.5.1 Land preparation

A fine tilth was made by thorough ploughing of the experimental field. The stubbles were removed and field was laid out as shown in plate 1. Lime was applied during first ploughing at the rate of 250 kg ha^{-1} based on the soil pH. Raised beds were prepared at a height of 30 cm with each plots having 2 m length and 1.5 m width. Each plots were separated by 40 cm space.

3.3.5.2 Manuring and fertilizer application

FYM was applied at the rate of 20 t ha^{-1} and thoroughly mixed before sowing. Macro nutrient fertilizers were applied as per POP recommendation for cowpea. Nitrogen was applied at the rate of 20 t ha^{-1} in two split doses first half at final ploughing and the next half at 15 DAS. Phosphorus and potassium were applied basally at the rate of 30 and 10 t ha^{-1} respectively. Micronutrient formulation was sprayed according to the treatments.

3.3.5.3 Seed rate and sowing

Seeds of cowpea variety PGCP – 6 (Pant lobia 3) released by Pantnagar Agricultural University were used in the experiment. It is a short duration cowpea variety with high yield potential. Seeds were sown in furrows taken in the bed at the rate of 40 kg ha $^{-1}$ with a spacing of 30 cm between rows and 15 cm between plants.

3.3.5.4 Irrigation and after cultivation

Crop was irrigated throughout the growth and hand weeding was done at regular intervals.

3.3.5.5 Plant protection

Nimbicidine was sprayed to control thrips during initial growth period. Copper oxychloride 50 WP was drenched against collar rot.

3.3.5.6 Harvesting

Pods were harvested at fully matured stage. First harvesting was done at 65 DAS.

3.4 BIOMETRIC OBSERVATIONS

Biometric observations like plant height, number of branches, stem diameter were taken at flower bud initiation and at harvest. Number of nodules per plant, fresh weight and dry weight of nodules and number of days taken for first flowering were recorded. Number of pods per plant, number of seeds per pod, pod weight per plant, length of pod, dry matter per plant and root CEC were taken at harvest.

3.4.1 Plant height (cm)

Plant height was measured from base of the pant to tip of main shoot at flower bud initiation stage and at harvest using a meter scale.

3.4.2 Number of branches

Number of branches were recorded at flower bud initiation stage and at harvest.

3.4.3 Stem diameter (cm)

Stem diameter was taken at flower bud initiation stage and at harvest using a measuring tape from five index plants and the average value was noted.

3.4.4 Number of nodules per plant

Number of nodules per plant was counted at 30 DAS. Three plants were randomly uprooted from each plot and it was then washed to remove the soil and the count was taken.

3.4.5 Fresh weight of nodules (g)

Fresh weight of nodules was taken soon after separation of the nodules from roots.

3.4.6 Dry weight of nodules (g)

The nodules were kept in petri plates according to treatments and placed in an oven for 5 days at 50 degree celcius. Dry weight was recorded when the weight attains a constant value.

3.4.7 Number of days taken for first flowering

It is the day at which first flower emergence take place from date of sowing.

3.4.8 Number of pods per plant

The number of pods obtained from five index plant from each plot were recorded and the average was taken to obtain the number of pods per plant.

3.4.9 Number of seeds per pod

Ten pods from index plants were selected randomly and the number of seeds from each pod were counted and the average was taken as the number of seeds per pod.

3.4.10 Pod weight per plant (g)

Total pod weight of ten plants selected as index plants were recorded and the average value was taken as the pod weight per plant.

3.4.11 Length of pod (cm)

Ten pods were randomly selected from the index plants and measured the length using a meter scale. The mean value was taken as the length of pod.

3.4.12 Grain yield (kg ha^{-1})

Total grain yield from each plot was recorded and the yield was calculated in kg per plot and yield in kg per hectare were calculated

3.4.12 Dry matter production (kg ha^{-1})

Five plants were uprooted from each plot and fresh weight was recorded. The plants were kept for shade drying and then oven dried at 65° C. Dry weight was recorded to find out the dry matter production per plant which was then used for calculating dry matter production in one hectare.

3.4.13 Root CEC

The CEC of root was determined as per the standard procedure given by Mitsui and Ueda in 1963. Roots were washed in distilled water and shaked with potassium chloride and titrated against NaOH.

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3.5 SOIL ANALYSIS

3.5.1 Initial soil analysis

Initial soil sample was collected from the prepared field for analysis at different places randomly and it is then pooled and reduced to 500 g and air dried. The air dried sample was sieved with 2 mm sieve and stored in an air tight container.

The soil samples were analyzed for pH, EC, organic carbon, CEC, bulk density, particle density, available macronutrients such as N, P, K and micronutrients such as Fe, Mn, Zn, Cu, B and Mo following the standard procedures in Table 1. Initial soil properties are given in Table 2.

3.5.2 Experimental soil analysis

Soil samples for laboratory analysis were collected from each plot at 45 DAS and at harvest. The samples were air dried, ground, sieved with 2 mm sieve and stored in air tight container. They were analyzed for pH, EC, organic carbon, available nutrients such as N, P, K, Fe, Mn, Zn, Cu, B and Mo as per the standard procedures as given in Table 1.

3.6 LEAF ANALYSIS

Leaf samples were collected at harvest and analyzed for nutrients such as N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B and Mo using standard procedures given in Table 3.

3.7 GRAIN ANALYSIS

Fully matured grains from each plots were collected and dried in an oven at 60 ° C and powdered for the analysis of N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B and Mo using standard procedures given in Table 3.

3.8 MICROBIOLOGICAL OBSERVATION

3.8.1 Soil biomass carbon

Soil biomass carbon was determined by fumigation extraction method as per the procedure put forward by Jenkinson and Poulson (1976). Fumigation of the collected soil samples were done with ethylene free chloroform in vacuum desiccator for 24 hours.

3.9 INCIDENCE OF PEST AND DISEASE

There was no severe incidence of pest and disease observed in the field. Crops were infested with thrips during initial growth period and was controlled by the application of nimbicidine. A few plants were infected with collar rot which was controlled by drenching of copper oxychloride.

3.10 BENEFIT COST RATIO

Benefit cost ratio was calculated using the formula

$$BCR = \frac{\text{Gross income}}{\text{Total expenditure}}$$

3.11 STATISTICAL ANALYSIS

The data obtained from the seed treatment study and field experiment were analyzed statistically using Analysis of Variance (ANOVA) for completely randomized design and factorial randomized block design proposed by Panse and Sukhatme in 1985. It was then tested for its significance using standard statistical tools.

Table 1 Analytical methods followed for soil analysis

Sl.No	Parameters	Method	Reference
1	pH	pH meter	Jackson (1958)
2	EC	Conductivity meter	Jackson (1958)
3	Organic carbon	Chromic acid wet digestion method	Walkley and Black (1934)
4	Bulk density	Undisturbed core sample	Black *et al.* (1965)
5	Textural analysis	International pipette method	Robinson (1922)
6	Available N	Alkaline permanganate method	Subbiah and Asija (1956)
7	Available P	Bray extraction and photoelectric colorimetry	Jackson (1958)
8	Available K	Flame photometry	Pratt (1965)
9	Available Fe	Atomic absorption spectroscopy	Sims and Johnson (1991)
10	Available Mn	Atomic absorption spectroscopy	Sims and Johnson (1991)
11	Available Zn	Atomic absorption spectroscopy	Emmel *et al.* (1977)
12	Available Cu	Atomic absorption spectroscopy	Emmel *et al.* (1977)
13	Available B	Photo electric colorimetry	Bingham (1982)
14	Available Mo	Inductively coupled plasma optical emission spectrometry (ICP-OES)	Soltanpour and Schwab (1977)

Table 2 Properties of initial soil sample

S.No	Parameter	Value
I. Physical properties		
1.	Bulk density (g cm^{-3})	1.32
2.	Particle density (g cm^{-3})	2.55
II. Textural composition		
1.	Sand (%)	77.4
2.	Silt (%)	19.6
3.	Clay (%)	3.75
4.	Textural class	Loamy sand
III. Chemical properties		
1.	pH (1:2.5)	4.36
2.	EC (dS m^{-1})	0.122
3.	Organic carbon (%)	0.65
4.	Organic matter (%)	1.11
5.	CEC (meq $100g^{-1}$)	7.45
6.	Available N (kg ha^{-1})	228.45
7.	Available P(kg ha^{-1})	41.2
8.	Available K(kg ha^{-1})	121.45
9.	Available Zn (mg kg^{-1})	0.98
10.	Available B (mg kg^{-1})	0.20
11.	Available Fe (mg kg^{-1})	65.34
12.	Available Cu (mg kg^{-1})	1.62
13.	Available Mn (mg kg^{-1})	12.30
14.	Available Mo (mg kg^{-1})	0.002

Table 3 Analytical methods followed for leaf and grain analysis

S.No	Parameter	Method	Reference
1.	Total N	Modified kjeldhal digestion method	Jackson (1958)
2.	Total P	Vanadomolybdate yellow colour method	Piper (1966)
3.	Total K	Flame photometry	Jackson (1958)
4.	Total Ca	Atomic absorption spectroscopy	Issac and Kerber (1971)
5.	Total Mg	Atomic absorption spectroscopy	Issac and Kerber (1971)
6.	Total S	Turbidimetric method	Bhargava and Raghupathi (1995)
7.	Total Zn	Atomic absorption spectroscopy	Emmel *et al.* (1977)
8.	Total B	Azomethane - H colorimetric method	Bingham (1982)
9.	Total Fe	Atomic absorption spectroscopy	Piper (1966)
10.	Total Cu	Atomic absorption spectroscopy	Emmel *et al.* (1977)
11.	Total Mn	Atomic absorption spectroscopy	Piper (1966)
13.	Total Mo	Inductively coupled plasma optical emission spectrometry (ICP-OES)	Soltanpur and Schwab (1977)

Plate 2 A: Seeds soaked in micronutrient formulation

Plate 2 B: Different stages of growth of seeds

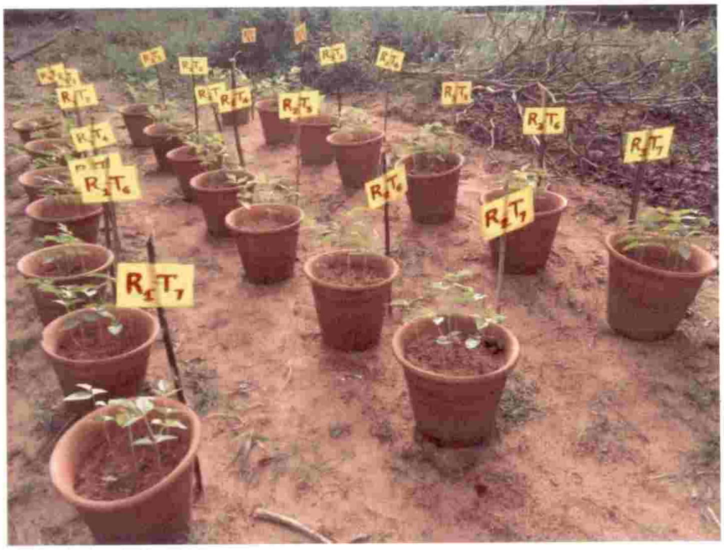


Plate 2 C: Cowpea plants at three leaf stage

Plate 2: Seed treatment study, COA, Padannakkad

Plate 3 A: Field layout

Plate 3 B: Bed preparation

Plate 3 C: Sowing of seeds

Plate 3 D: Germination stage

Plate 3 E: Crop at 15 DAS

Plate 3 F: Crop at 30 DAS

Plate 3: Experimental plot at RARS, Pilicode

Plate 4 A: Flowering and pod formation

Plate 4 B: Harvesting of pods

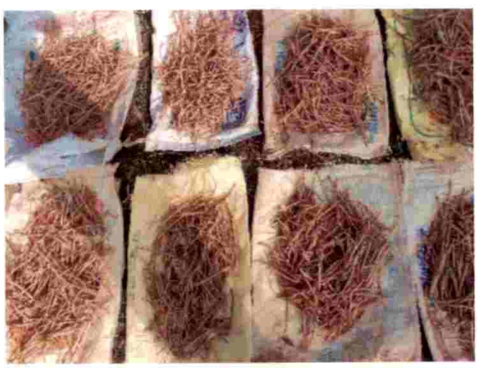


Plate 4 C: Harvested pods

Plate 4 D: Cowpea grain

Plate 4 E: Field at harvest stage

Plate 4: Experimental plot at harvest stage

Results

4. RESULTS

The results obtained from the experiment entitled "Evaluation of micronutrient formulation in cowpea (*Vigna unguiculata* L. Walp)" was carried out in RARS, Pilicode. The data obtained were subjected to statistical analysis and the results are given in this chapter.

4.1 STANDARDIZATION OF MICRONUTRIENT FORMULATION FOR COWPEA

A micronutrient mixture in liquid form was formulated and standardized for tissue culture banana by Premalatha (2016), an MSc student at COA Padannakkad. Later it was standardized for bhindi by Ashwini (2018) as part of her MSc research work. The mixture is a combination of two solutions A and B in which solution A contains a mixture of micronutrient salts namely zinc sulphate (10 %), boric acid (2 %), copper sulphate (4 %), manganous sulphate (0.1 %), ferrous sulphate (2 %) and ammonium molybdate (0.05 %). Solution B is an organic chelating agent. The objective of this experiment was to standardize the micronutrient formulation for cowpea in seed treatment as well as foliar spray. The micronutrient mixture standardized for vegetables showed less shelf life and forms precipitate when solution A and solution B were mixed together. This was found to be the major drawback of the formulation for banana and vegetables. Several trials were conducted to make a formulation having no precipitate formation while mixing solutions A and B. Thus at a specific combination of solution A and Solution B the mixture was found to be stable and tested for its suitability on cowpea as foliar spray (Plate 4).

Composition of Solution A (1 litre)

$ZnSO_4.7H_2O$	- 50 g
$CuSO_4.5H_2O$	- 20 g
$FeSO_4.7H_2O$	- 10 g

H_3BO_3 - 10 g

$MnSO_4.H_2O$ - 0.5 g

$(NH_4)_6Mo_7O_{24}.4H_2O$ - 0.5 g

4.2 SEED TREATMENT STUDY

4.2.1 Standardization of time and dose of micronutrient formulation for seed treatment of cowpea

Micronutrient mixture at different concentrations at the rate of 0.25, 0.50, 0.75, 1, 1.5 and 2 percentages were used for seed treatment study in cowpea. To standardize the time for seed treatment several trials were conducted. Cowpea seeds were treated with micronutrient mixture both at different concentrations of micronutrient mixture for different durations (1 hr, 2hrs, 4 hrs, 6hrs, 8 hrs, 10 hrs and 12 hrs). Observations on seed germination and its emergence showed that the seeds treated with micronutrient mixture for 6 hours showed better establishment as compared to other duration of seed treatment. In the case of cowpea seeds treated for longer duration, the germination and seedling emergence was adversely affected (Plate 5). Thus time and concentration of micronutrient mixture for cowpea seed treatment were standardized. Based on this a seed treatment study was carried out in cowpea and the following biometric observations were recorded.

4.2.2 Biometric observations from seed treatment study

4.2.2.1 Germination percentage (%)

The data obtained on the germination percentage of cowpea seeds is given in the Table 4. The observations were found to be non significant in all the treatments with 100 per cent germination occurred in T_5 (micronutrient mixture @ 1.5 %) and T_6 (micronutrient mixture @ 2 %).

4.2.2.2 Days taken for germination

Number of days taken for the germination of cowpea seeds are given in the Table 4. Treatments did not show significant difference in the days taken for germination.

4.2.2.3 Seedling length at three leaf stage (cm)

Seedling length at three leaf stage is given in the Table 4. Treatment T_6 (24.23) showed maximum seedling length followed by T_5 (22.31) and there was significant difference between the two treatments. Control recorded the lowest value in T_7 (18.75).

4.2.2.4 Seedling vigour index

Seedling vigour index data obtained from seed treatment study is given in the Table 4. Treatment T_6 (2423) showed highest seedling vigour index which was significantly superior to all other treatments. Treatment T_5 showed the second highest seedling vigour index (2231) and the lowest value was recorded in control (1814.25).

The observations of seed treatment study showed that the treatment T_6 (micronutrient mixture @ 2 %) was identified as the best treatment and T_5 (micronutrient mixture @ 1.5 %) as the second best treatment for evaluating the performance of micronutrient formulation in field trial of cowpea.

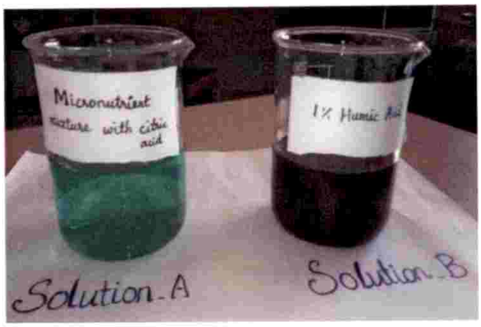


Plate 5 A: Micronutrient mixture for TC banana

Plate 5 B: Precipitate formation on mixing Solution A and B

Plate 5 C: Solution A with Solution B without precipitation

Plate 5: Preparation of micronutrient mixture for cowpea

1 hour

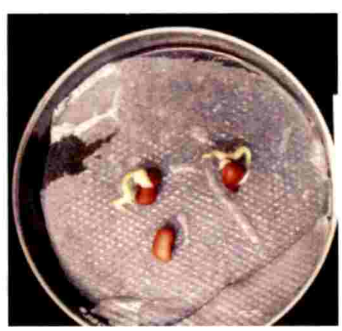
2 hours

4 hours

6 hours

8 hours

10 hours



12 hours

Plate 6: Standardization of time for seed treatment in cowpea using 2% mixture

Table 4: Effect of seed treatment with micronutrient formulation on germination percentage, days taken for germination, seedling length at three leaf stage and seedling vigour index

Treatment	Germination percentage (%)	Days taken for germination	Seedling length at three leaf stage (cm)	Seedling vigour index
T_1	96.70	1.66	18.80	1817.96
T_2	96.76	1.66	19.81	1881.95
T_3	97.77	1.67	19.84	1939.75
T_4	97.77	1.66	19.94	1949.53
T_5	100.00	1.33	22.31	2231.00
T_6	100.00	1.00	24.23	2423.00
T_7	95	1.77	18.75	1814.25
SE(m)	2.22	0.28	0.40	45.38
C.D.	NS	NS	1.24	138.99

4.3 FIELD EXPERIMENT

4.3.1 Observations on growth and yield characters of cowpea

4.3.1.1 Plant height (cm)

The data on plant height was recorded at the time of flower bud initiation and at harvest. The effect of seed treatment, foliar spray and their interactions on plant height is recorded in the Table 5.

Levels of seed treatment showed significant difference in plant height at flower bud initiation and at harvest. At flower bud initiation S_3 (seed treatment with 2 % micronutrient formulation) recorded maximum plant height (24.31) which was on par with seed treatment with 1.5 % micronutrient formulation, S_4 (23.53). At the time of harvest S_3 recorded maximum height of 39.75 cm which was significantly superior to all other treatments.

Levels of foliar spray of micronutrients showed significant difference in plant height at flower bud initiation and at harvest. Maximum height was observed in F_3 treatment (foliar spray at 15 DAS and 30 DAS) both at flower bud initiation (25.98) and at harvest (41.08).

In the interaction effect maximum plant height was recorded in the treatment S_3F_3 (seed treatment with 2 % micronutrient formulation + two foliar spray) at both the stages given as 29.80 and 44.13, which were significantly different from all other treatments.

4.3.1.2 Number of branches

The data on number branches recorded at flower bud initiation and at harvest with respect to various treatments are recoded in the Table 5.

Significant difference was not observed in the number of branches at flower bud initiation and at harvest in seed treatment, foliar spray and their interaction.

4.3.1.3 Stem diameter (cm)

There was significant difference in stem diameter among the treatments. The results are recorded in the Table 5.

Stem diameter showed significant difference with variation in seed treatment. Maximum diameter was observed in treatment S_4 (2.44) at flower bud initiation which was on par with S_3 (2.43) and seed treatment with rhizobium, S_2 (2.35). At the time of harvest treatment S_3 recorded the highest stem diameter (4.65) which was significantly different from all other treatments.

Levels of foliar spray showed significant difference in stem diameter at flower bud initiation. F_3 recorded maximum stem diameter (2.54) which was on par with single spray of micronutrient formulation, F_2 (2.40). At the time of harvest, F_3 (4.85) recorded maximum stem diameter which was significantly different from all other treatments.

Interaction effect of treatments also showed significant difference among the treatments. At flower bud initiation maximum stem diameter was recorded in seed treatment with rhizobium + two foliar spray, S_2F_3 (2.86) which was on par with seed treatment with 2 % micronutrient formulation + single foliar spray, S_3F_2 (2.66), seed treatment with 1.5 % micronutrient formulation + two foliar sprays, S_4F_3 (2.64) and seed treatment with 1.5 % micronutrient formulation + one foliar spray, S_4F_2 (2.62). At harvest S_2F_3 (5.30) showed maximum stem diameter which showed significant difference from all other treatments.

4.3.1.4 Number of nodules per plant

The data on number of nodules per plant are presented in the Table 6.

Levels of seed treatment showed significant difference in the number of nodules per plant. Maximum nodules per plant was observed in plots treated with rhizobium, S_2 (27.22). The least number of nodules was observed in no seed treatment, S_1 (14.77) which was on par with S_3 (18.88) and S_4 (19.66).

There was no considerable difference in the number of nodules with respect to foliar spray and the interaction effect of seed treatment and foliar spray. The results obtained was found to be non significant.

4.3.1.5 Fresh weight of nodules (g)

The effect of seed treatment, foliar spray and their interaction on fresh weight of nodules are given in the Table 6. Effect of seed treatment, foliar spray and their interaction on fresh weight of nodules was found to be non significant.

4.3.1.6 Dry weight of nodules (g)

The effect of seed treatment, foliar spray and their interaction on dry weight of nodules are given in the Table 6. Effect of seed treatment, foliar spray and their interaction on dry weight of nodules was found to be non significant

Table 5: Effect of seed treatment, foliar spray and their interactions on plant height, number of branches and stem diameter of cowpea (at flower bud initiation stage and harvest)

Treatment	Plant height (cm)		Number of branches plant^{-1}		Stem diameter plant^{-1} (cm)	
Levels of seed treatment	Flower bud initiation	Harvest	Flower bud initiation	Harvest	Flower bud initiation	Harvest
S_1	20.86	35.97	9.87	9.97	2.11	4.16
S_2	23.11	38.41	9.89	9.99	2.35	4.58
S_3	24.31	39.75	10.34	10.45	2.43	4.65
S_4	23.53	38.63	10.38	10.40	2.44	4.26
SEm (±)	0.27	0.35	0.17	0.16	0.06	0.01
CD (0.05)	0.81	1.05	NS	NS	0.17	0.05
Levels of foliar spray						
F_1	21.26	36.57	9.85	9.89	2.06	3.91
F_2	21.61	36.92	10.12	10.15	2.40	4.49
F_3	25.98	41.08	10.10	10.15	2.54	4.85
SEm (±)	0.23	0.30	0.15	0.15	0.05	0.01
CD (0.05)	0.70	0.90	NS	NS	0.15	0.04
Interactions						
S_1F_1	18.66	34.33	9.87	9.98	1.73	3.58
S_1F_2	21.73	36.40	9.88	9.89	2.32	4.58
S_1F_3	22.20	37.20	9.99	9.98	2.29	4.34
S_2F_1	21.93	36.60	10.11	10.12	2.19	4.38
S_2F_2	21.40	36.63	10.13	10.14	2.02	4.06
S_2F_3	26.00	42.00	10.11	10.12	2.86	5.30
S_3F_1	21.06	37.73	9.98	9.99	2.24	4.38
S_3F_2	22.06	37.40	9.97	9.98	2.66	4.72
S_3F_3	29.80	44.13	10.12	10.13	2.39	4.86
S_4F_1	23.40	37.63	10.12	10.13	2.07	3.30
S_4F_2	21.26	37.26	9.98	9.99	2.62	4.60
S_4F_3	25.93	41.00	9.99	10.00	2.64	4.90
SEm (±)	0.47	0.61	0.30	0.30	0.10	0.02
CD (0.05)	1.40	1.81	NS	NS	0.30	0.08

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

4.3.1.7 Number of days taken for first flowering

The data on days taken for first flowering of cowpea as influenced by seed treatment, levels of foliar application of micronutrients and their interactions are given in Table 6. Main treatments as well as their interactions did not show any significant difference in the number of days taken for first flowering. However, the minimum days for flowering in main treatments were observed as 31 days both in S_3 and F_2 treatments. The interaction effect was also non significant with maximum days (32 days) in control.

4.3.1.8 Number of pods per plant

The data on number of pods per plant influenced by seed treatment, levels of foliar application of micronutrients along with their interactions are given in Table 7.

Levels of seed treatment showed significant difference in the number of pods per plant with maximum number recorded in S_3 (21.02) which was on par with S_4 (19.44). The lowest number of pods per plant was observed in S_1 (16.73).

Levels of foliar spray showed significant difference in the number of pods per plant with maximum number recorded in F_3 (20.97). F_1 recorded the lowest number (16.71).

The interaction effect also showed significant difference in number of pods per plant with maximum number recorded in S_3F_3 (25.84) which was on par with seed treatment with 1.5 % micronutrient formulation + two foliar sprays, S_4F_3 (21.01). S_1F_1 (16) recorded the least value.

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Table 6: Effect of seed treatment, foliar spray and their interactions on number of nodules per plant, fresh weight of nodules, dry weight of nodules and number of days taken for first flowering of cowpea

Treatment	Number of nodules $plant^{-1}$	Fresh weight of nodules (g)	Dry weight of nodules (g)	Number of days taken for first flowering
Levels of seed treatment				
S_1	14.77	0.21	0.16	32.11
S_2	27.22	0.39	0.33	31.20
S_3	18.88	0.40	0.34	31.00
S_4	19.66	0.47	0.41	31.11
SEm (±)	1.93	0.08	0.08	0.19
CD (0.05)	5.71	NS	NS	NS
Levels of foliar spray				
F_1	17.29	0.28	0.23	32.00
F_2	20.37	0.37	0.31	31.25
F_3	22.75	0.45	0.39	31.41
SEm (±)	1.67	0.07	0.07	0.16
CD (0.05)	NS	NS	NS	NS
Interactions				
S_1F_1	12.83	0.10	0.07	32.33
S_1F_2	15.66	0.27	0.22	31.33
S_1F_3	15.83	0.26	0.20	31.00
S_2F_1	25.66	0.36	0.30	31.00
S_2F_2	24.66	0.33	0.27	32.00
S_2F_3	31.33	0.47	0.41	30.00
S_3F_1	14.00	0.25	0.19	30.66
S_3F_2	20.66	0.41	0.36	31.00
S_3F_3	22.00	0.53	0.48	30.00
S_4F_1	16.66	0.40	0.34	31.00
S_4F_2	20.50	0.46	0.40	31.66
S_4F_3	21.83	0.54	0.48	31.41
SEm (±)	3.35	0.14	0.14	0.33
CD (0.05)	NS	NS	NS	NS

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

4.3.1.9 Number of seeds per pod

The effect of seed treatment, foliar spray of micronutrient mixture and their interaction on number of seeds per pod is given in Table 7. Treatments did not show any significant difference in the number of seeds per pod.

4.3.1.10 Pod weight per plant (g)

The effect of seed treatment, micronutrient foliar spray and their interaction on pod weight per plant is given in Table 7.

Levels of seed treatment showed significant difference in pod weight per plant. S_3 recorded highest pod weight per plant (36.14) which was on par with S_4 (31.90). It was then followed by S_2 (29.73) and control recorded the least (24.92).

Levels of foliar spray showed significant difference in pod weight per plant with F_3 (33.49) recorded the highest value which was superior over all other treatments. The treatment F_1 showed lowest value (26.43).

Interaction effect of seed treatment and foliar spray of micronutrient in pod weight was found to be significant with S_3F_3 (38.11) recorded maximum pod weight per plant which was on par with S_4F_3 (31.23). Treatment S_1F_1 (24.64) recorded the lowest value.

4.3.1.11 Length of pod (cm)

Observations obtained on the length of pod per each treatment is given in Table 7. Length of pod did not show any significant difference in the main treatments as well as their interactions.

4.3.1.12 Grain yield (kg ha^{-1})

Grain yield of cowpea with respect to seed treatment, foliar spray and their interaction is given in the Table 7.

Levels of seed treatment showed significant difference in yield with highest value was obtained in S_3 (2224) superior over all other seed treatments. Lowest yield was obtained in S_1 (1969).

Levels of foliar spray showed significant difference in yield with highest was recorded in F_3 (2173) which was superior over all other treatments and the lowest yield was recorded in F_1 (1979).

Interaction of seed treatment and foliar spray also showed significant difference in the yield with highest was recorded in S_3F_3 (2320) which was superior over other treatment interactions. It was followed by S_3F_2 (2220) which was on par with S_4F_3 (2200). Lowest yield was recorded in S_1F_1 (1820).

4.3.1.13 Dry matter production (kg ha^{-1})

Dry matter production per plant as obtained from seed treatment, foliar spray of micronutrient and their interaction is given in the Table 7. Dry matter production recorded was maximum in S_3 (2292.91) which was superior over other seed treatments. Dry matter production was lowest in S_1 (2000.06). Foliar spray F_3 showed maximum dry matter production per plant (2372.92) which was superior over other levels of foliar spray. Interaction of main treatments showed significant difference in dry matter production with S_3F_3 (2613.24) produced highest amount which was superior over other interactions.

4.3.1.14 Root CEC (cmol kg^{-1})

Results obtained on root CEC is given in Table 7. Root CEC was found to be significant with respect to different treatments. Among the seed treatment root CEC was found to be highest in S3 (6.00) and in F3 (5.87) of foliar spray. In the interaction effect S3F3 (6.13) recorded the highest value which was on par with S2F3 (5.98).

X

Table 7: Effect of seed treatment, foliar spray and their interactions on number of pods per plant, number of seeds per pod, pod weight per plant, length of pod, dry matter production and root CEC

Treatment	No of pods plant^{-1}	No. of seeds pod^{-1}	Pod weight plant^{-1} (g)	Length of pod (cm)	Grain yield (kg ha^{-1})	Dry matter (kg ha^{-1})	Root CEC (cmol kg^{-1})
Levels of seed treatment							
S_1	16.73	15.46	24.92	17.64	1969	2000.06	5.58
S_2	17.97	15.73	29.73	17.63	1977	2172.62	5.66
S_3	21.02	15.13	36.14	18.11	2224	2292.91	6.00
S_4	19.44	14.80	31.90	17.94	2168	2220.94	5.88
SEm (±)	0.91	0.43	2.28	0.39	16	16.37	0.03
CD (0.05)	2.68	NS	4.26	NS	48	48.33	0.10
Levels of foliar spray							
F_1	16.71	15.53	26.43	17.85	1979	2042.53	5.72
F_2	18.63	14.93	27.80	17.85	2101	2099.46	5.76
F_3	20.97	15.38	33.49	17.80	2173	2372.92	5.87
SEm (±)	0.78	0.37	0.79	0.34	14	14.18	0.03
CD (0.05)	2.32	NS	2.33	NS	41	41.85	0.09
Interactions							
S_1F_1	16.00	15.46	24.64	17.26	1820	1804.07	5.43
S_1F_2	16.67	15.33	25.00	17.50	2000	2068.19	5.60
S_1F_3	15.53	15.60	25.91	18.16	2113	2127.92	5.73
S_2F_1	13.40	15.80	26.00	17.60	1843	2037.39	5.56
S_2F_2	18.53	15.26	26.51	17.93	2000	2193.57	5.67
S_2F_3	20.20	16.13	30.00	17.36	2059	2286.90	5.98
S_3F_1	18.40	15.53	28.32	18.66	2133	2104.28	5.45
S_3F_2	19.67	14.73	29.98	18.00	2220	2161.21	5.82
S_3F_3	25.00	15.13	38.11	17.66	2320	2613.24	6.13
S_4F_1	17.06	15.33	27.24	17.86	2120	2224.37	5.80
S_4F_2	19.93	14.40	28.00	17.96	2186	1974.86	5.86
S_4F_3	21.01	14.66	31.23	18.00	2200	2463.60	5.92
SEm (±)	1.57	0.75	2.38	0.69	28	28.36	0.06
CD (0.05)	4.72	NS	7.15	NS	83	83.71	0.18

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

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4.4 SOIL NUTRIENT ANALYSIS

4.4.1 Soil pH

The effect of seed treatment, foliar spray and their interaction on soil pH at 45 days after sowing and at harvest is given in the Table 8. Soil pH was found to be non significant in both 45 days after sowing and at harvest. The lowest pH at 45 days after sowing and at harvest was found in S_3 (4.43 and 4.39), F_3 (4.49 and 4.42) and S_2F_1 (4.33 and 4.37).

4.4.2 EC (dSm^{-1})

Electrical conductivity of soil was not affected by the treatments at both 45 days after sowing and at harvest. The readings are given in the Table 8. Electrical conductivity was found to be higher in S_3 at both stages (0.12 and 0.14), F_3 (0.10 and 0.13) and S_3F_3 (0.12 and 0.14).

4.4.3 Organic carbon (%)

The effect of seed treatment, foliar spray of micronutrient mixture and their interaction is given in the Table 8.

Organic carbon was found to be non significant in seed treatment both at 45 days after sowing and at harvest.

Levels of foliar spray of micronutrient mixture recorded significant difference in organic carbon content at harvest with F_3 (0.63) superior over all other treatments. At 45 days after sowing organic carbon was non significant with respect to different levels of foliar spray.

Interaction effect of treatments were found to be non significant at 45 days after sowing. At harvest organic carbon content of soil was found to be the highest in S_3F_3 (0.69) which was on par with S_2F_3 (0.66), S_4F_3 (0.64), S_4F_1 (0.63), S_4F_2 (0.62) and S_3F_2 (0.62).

Table 8: Effect of seed treatment, foliar spray and their interactions on soil pH, EC and organic carbon at 45 DAS and harvest

Treatment	pH		EC (dSm^{-1})		Organic carbon (%)	
Levels of seed treatment	45 DAS	Harvest	45 DAS	Harvest	45 DAS	Harvest
S_1	4.54	4.50	0.11	0.13	1.12	0.60
S_2	4.44	4.42	0.08	0.11	1.10	0.60
S_3	4.43	4.39	0.12	0.14	1.11	0.63
S_4	4.44	4.41	0.09	0.11	1.12	0.63
SEm (±)	0.08	0.08	0.01	0.01	0.009	0.06
CD (0.05)	NS	NS	NS	NS	NS	NS
Levels of foliar spray						
F_1	4.61	4.66	0.10	0.12	1.11	0.58
F_2	4.60	4.61	0.10	0.12	1.11	0.59
F_3	4.49	4.42	0.10	0.13	1.12	0.63
SEm (±)	0.07	0.07	0.01	0.011	0.008	0.014
CD (0.05)	NS	NS	NS	NS	NS	0.04
Interactions						
S_1F_1	4.47	4.51	0.08	0.14	1.12	0.53
S_1F_2	4.62	4.63	0.11	0.14	1.13	0.55
S_1F_3	4.53	4.57	0.0	0.12	1.12	0.54
S_2F_1	4.33	4.37	0.09	0.12	1.10	0.56
S_2F_2	4.51	4.55	0.08	0.11	1.10	0.57
S_2F_3	4.49	4.53	0.08	0.11	1.12	0.66
S_3F_1	4.75	4.80	0.10	0.12	1.13	0.61
S_3F_2	4.62	4.66	0.08	0.10	1.11	0.62
S_3F_3	4.76	4.80	0.12	0.14	1.11	0.69
S_4F_1	4.81	4.86	0.11	0.13	1.10	0.63
S_4F_2	4.67	4.71	0.09	0.11	1.13	0.6
S_4F_3	4.68	4.72	0.12	0.14	1.13	0.64
SEm (±)	0.14	0.14	0.02	0.02	0.015	0.02
CD (0.05)	NS	NS	NS	NS	NS	0.071

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

4.4.4 Available nitrogen in soil (kg ha $^{-1}$)

The effect of treatments and their interaction on the available nitrogen present in the soil is given in the Table 9.

Levels of seed treatment showed considerable difference in the amount of available nitrogen at 45 days after sowing and harvest. At both stages S_2 (seed treatment with rhizobium) showed highest nitrogen content in soil compared to other treatments which were significantly different from other treatments (384.43 and 355.63). S_1 (no seed treatment) showed the lowest value at both stages (362.94 and 346.69).

Levels of foliar spray and the interaction effect on available nitrogen were found to be non significant at 45 days after sowing and at harvest.

4.4.5 Available phosphorus in soil (kg ha $^{-1}$)

The data obtained on the available phosphorus content affected by seed treatment, foliar spray of micronutrient mixture and their interaction are given in the Table 9.

Levels of seed treatment showed significant difference in the available P content at 45 days after sowing and at harvest with values recorded as 61.67 and 45.11 in S_2 (seed treated with rhizobium) which was superior over all other treatments.

Phosphorus content at both stages were found to be non significant at different levels of foliar spray as well as interactions.

4.4.6 Available potassium in soil (kg ha $^{-1}$)

Available K content in soil affected by various treatments and their interaction at 45 days after sowing and at harvest is given in the Table 9.

Available K content was highest in S_3 at both 45 days after sowing and at harvest (220.66 and 170.67). F_3 recorded highest available potassium in both stages

(218.16 and 168.33). Interaction effect of main treatments showed maximum value S_3F_3 at 45 days after sowing and at harvest as 229.38 and 179.00 respectively.

Table 9: Effect of seed treatment, foliar spray and their interactions on available N, P, and K in soil

Treatment	Available nitrogen (kg ha $^{-1}$)		Available phosphorus (kg ha $^{-1}$)		Available potassium (kg ha $^{-1}$)	
Levels of seed treatment	45 DAS	Harvest	45 DAS	Harvest	45 DAS	Harvest
S_1	362.94	346.69	54.74	33.33	195.55	145.66
S_2	384.43	355.63	61.76	45.11	202.66	152.66
S_3	367.92	349.56	54.54	35.74	220.66	170.67
S_4	364.70	346.34	54.15	34.26	211.56	161.77
SEm (±)	1.86	1.86	0.41	0.26	0.07	0.05
CD (0.05)	5.51	5.50	1.23	0.79	0.22	0.16
Levels of foliar spray						
F_1	367.05	356.61	56.29	36.73	200.25	150.25
F_2	372.72	362.28	56.03	37.36	204.41	154.50
F_3	370.22	359.78	56.57	37.25	218.16	168.33
SEm (±)	1.61	1.61	0.36	0.23	0.06	0.04
CD (0.05)	NS	NS	NS	NS	0.22	0.14
Interactions						
S_1F_1	359.98	343.73	54.56	33.00	185.00	135.23
S_1F_2	364.48	348.23	54.77	34.00	189.66	140.32
S_1F_3	364.36	348.11	54.90	33.00	212.00	162.44
S_2F_1	378.45	379.65	61.00	44.14	196.09	146.50
S_2F_2	393.08	394.28	62.29	45.21	200.01	150.00
S_2F_3	381.76	372.96	62.00	46.00	212.00	162.30
S_3F_1	366.92	348.56	54.80	35.10	215.09	165.23
S_3F_2	367.69	349.33	53.02	36.12	218.02	168.09
S_3F_3	369.15	350.79	55.80	36.00	229.38	179.00
S_4F_1	362.87	344.51	54.80	34.70	205.02	155.33
S_4F_2	365.63	347.27	54.06	34.10	210.02	160.22
S_4F_3	365.61	347.25	53.60	34.00	219.66	170.33
SEm (±)	3.23	3.22	0.36	0.46	0.13	0.09
CD (0.05)	NS	NS	NS	NS	0.38	0.28

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

4.4.7 Available iron in soil (mg kg^{-1})

The data recorded on the available iron content affected by seed treatment, foliar spray of micronutrient mixture and their interaction is given in the Table 10.

At 45 days after sowing available iron content was found to be non significant with respect to various levels of seed treatment. At harvest S_3 recorded highest value (55.22) which was on par with S_4 (54.69).

Levels of seed treatment showed significant difference in available iron content. F_3 showed maximum iron content at 45 days after sowing and at harvest (55.50 and 51.28) which was superior than other treatments.

Interaction effect of seed treatment and foliar spray also showed significant difference in available iron content. Iron content was found to be the highest in S_1F_3 (87.66) at 45 days after sowing and S_3F_3 (60.94) at harvest.

4.4.8 Available manganese in soil (mg kg^{-1})

The data on available manganese at 45 days after sowing and at harvest with respect to seed treatment, foliar spray and their interaction is given in the Table 10.

Seed treatment did not show any significant difference in available manganese content at 45 days after sowing. At harvest manganese content showed significant difference with highest content recorded in S_3 (21.71) which was on par with S_4 (21.37) and S_2 (18.78).

Levels of foliar spray showed significant difference among the treatments with highest value recorded in F_3 (32.72 and 21.83) at both stages and superior over all other treatments.

Interaction between the treatments also showed significant difference with highest value recorded in S_3F_3 with 37.57 and 26.68 at 45 days after sowing and at harvest respectively.

Table 10: Effect of seed treatment, foliar spray and their interactions on available Fe, Mn and Zn in soil

Treatment	Available Fe ($mg\ kg^{-1}$)		Available Mn ($mg\ kg^{-1}$)		Available Zn ($mg\ kg^{-1}$)	
Levels of seed treatment	45 DAS	Harvest	45 DAS	Harvest	45 DAS	Harvest
S_1	82.34	50.34	27.41	16.53	4.16	1.68
S_2	81.54	49.35	26.67	18.78	4.56	1.73
S_3	82.36	55.22	32.54	21.71	4.59	1.77
S_4	82.51	54.69	32.26	21.37	4.56	1.76
SEm (±)	0.63	0.41	0.02	0.97	0.02	0.01
CD (0.05)	NS	1.23	NS	2.94	NS	0.03
Levels of foliar spray						
F_1	81.96	50.43	27.80	16.92	4.26	1.69
F_2	81.30	51.28	28.64	17.80	4.32	1.74
F_3	83.31	55.50	32.72	21.83	5.03	1.76
SEm (±)	0.55	0.36	0.02	0.02	0.02	0.01
CD (0.05)	1.62	1.07	0.07	0.07	0.07	0.03
Interactions						
S_1F_1	78.69	46.69	23.77	12.88	3.46	1.79
S_1F_2	80.68	48.69	25.69	14.80	3.76	1.80
S_1F_3	87.66	55.66	32.78	21.89	5.26	1.60
S_2F_1	83.69	51.69	28.93	18.04	4.46	1.77
S_2F_2	79.69	47.69	24.82	13.93	4.06	1.68
S_2F_3	81.23	48.69	26.26	15.37	4.26	1.88
S_3F_1	82.69	50.68	28.26	17.37	4.66	1.80
S_3F_2	83.72	54.05	31.79	21.07	4.75	1.80
S_3F_3	80.68	60.94	37.57	26.68	5.56	1.70
S_4F_1	82.78	52.69	30.26	19.37	4.46	1.70
S_4F_2	81.10	54.69	32.26	21.37	4.75	1.71
S_4F_3	83.66	56.70	34.26	23.37	5.06	1.88
SEm (±)	1.10	0.72	0.04	0.04	0.01	0.02
CD (0.05)	3.24	2.14	0.14	0.14	0.03	NS

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

4.4.9 Available zinc in soil (mg kg^{-1})

Effect of seed treatment, foliar application of micronutrient and their interaction on available zinc content at 45 days after sowing and at harvest is given in the Table 10.

Available zinc content was found to be non significant in seed treatment at 45 days after sowing. Zinc content showed significant difference at harvest in S_3 (1.77) which recorded the highest value which was on par with S_4 (1.76).

Levels of foliar spray showed significant difference in available zinc content with highest value recorded in treatment F_3 (5.03) at 45 days after sowing which was superior over other treatments and at harvest F_3 recorded the highest value (1.76) which was on par with F_2 (1.74).

Interaction showed significant difference in zinc content at 45 days after sowing with highest value obtained in S_3F_3 (5.56) which was found to be superior over other treatments and at harvest available zinc was non significant with respect to interactions.

4.4.10 Available copper in soil (mg kg^{-1})

The data obtained on available copper content at 45 days after sowing and at harvest effected by seed treatment, foliar spray and their interaction is given in the Table 11.

Levels of seed treatment showed no significant difference in copper content at 45 days after sowing and at harvest.

Levels of foliar spray showed significant difference in available copper content at 45 days after sowing and at harvest with F_3 superior over other treatments (3.57 and 2.69).

Interaction effect on copper content was found to be non significant at 45 days after sowing but was significant at harvest with higher value obtained in S4F3 (3.51).

4.4.11 Available boron in soil (mg kg^{-1})

The data on available boron at 45 days after sowing and at harvest with respect to treatments are given in the Table 11.

Available boron was highest in S_3 (0.59) at 45 days after sowing and non significant at harvest stage. Considering foliar spray of micronutrient mixture available boron was highest in F_3 (0.62) which was superior over other foliar sprays at 45 days after sowing and was non significant at harvest. Interaction effect showed significant difference in boron content with highest value obtained in S_3F_3 (0.71) which was superior over other treatments at 45 days after sowing. At harvest interaction effect did not show significant difference among treatments.

4.4.12 Available molybdenum in soil (mg kg^{-1})

The result obtained on available molybdenum content in soil at different stages is given in the Table 11.

There was no significant difference among the seed treatments, foliar spray and interaction at 45 days after sowing. At harvest S_3 (0.008) and F_3 (0.008) was found to be superior among main treatments. Interaction effect did not show any significant difference in available molybdenum content in soil at harvest.

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Table 11: Effect of seed treatment, foliar spray and their interactions on available copper, boron and molybdenum in soil

Treatment	Available Cu (mg kg^{-1})		Available B (mg kg^{-1})		Available Mo (mg kg^{-1})	
Levels of seed treatment	45 DAS	Harvest	45 DAS	Harvest	45 DAS	Harvest
S_1	3.35	1.81	0.51	0.25	0.005	0.004
S_2	3.48	1.88	0.49	0.24	0.006	0.005
S_3	3.52	2.93	0.59	0.25	0.005	0.008
S_4	3.48	2.63	0.57	0.26	0.007	0.006
SEm (±)	0.06	0.02	0.004	0.003	0.00013	0.00004
CD (0.05)	NS	NS	0.013	NS	NS	0.00012
Levels of foliar spray						
F_1	3.38	2.11	0.50	0.25	0.006	0.005
F_2	3.42	2.13	0.51	0.25	0.006	0.004
F_3	3.57	2.69	0.62	0.26	0.009	0.008
SEm (±)	0.05	0.04	0.004	0.002	0.00014	0.00006
CD (0.05)	0.15	0.12	0.011	NS	NS	0.00018
Interactions						
S_1F_1	3.31	1.31	0.44	0.25	0.003	0.002
S_1F_2	3.41	1.61	0.50	0.26	0.004	0.003
S_1F_3	3.33	2.51	0.60	0.25	0.007	0.006
S_2F_1	3.36	2.11	0.52	0.25	0.004	0.003
S_2F_2	3.40	1.51	0.46	0.24	0.005	0.004
S_2F_3	3.69	2.01	0.51	0.25	0.008	0.007
S_3F_1	3.53	2.41	0.52	0.26	0.009	0.008
S_3F_2	3.37	2.87	0.54	0.26	0.008	0.007
S_3F_3	3.66	2.75	0.71	0.25	0.010	0.009
S_4F_1	3.31	2.61	0.53	0.26	0.006	0.005
S_4F_2	3.51	2.55	0.54	0.25	0.005	0.003
S_4F_3	3.61	3.51	0.66	0.26	0.009	0.009
SEm (±)	0.10	0.08	0.008	0.004	0.00003	0.0005
CD (0.05)	NS	0.25	0.023	NS	NS	NS

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

4.5 TOTAL NUTRIENT CONTENT IN LEAF

4.5.1 Nitrogen (%)

Nitrogen concentration in the leaf at harvest is given in the Table 12. Effect of seed treatment on nitrogen content was non significant with highest value obtained in S_2 (2.07). Among the foliar spray F_3 (3.08) recorded highest nitrogen content in leaf at harvest which was superior over all other levels of foliar spray. Interaction of main treatments showed highest nitrogen concentration in S_3F_3 (3.70) which was on par with S_4F_3 (3.61).

4.5.2 Phosphorus (%)

Effect of treatments on the phosphorus content of leaf of cowpea is given in the Table 12. Seed treatment showed significant difference in phosphorus content with maximum in S_3 (0.26) which was superior over other treatments. It was significant in foliar spray at harvest with F_3 (0.25) which showed highest phosphorus content in leaf. S_3F_3 (0.28) showed highest value which was superior over other treatments.

4.5.3 Potassium (%)

Potassium content in leaf of cowpea at harvest is given in the Table 12. Seed treatments showed significant difference in potassium content and S_3 (1.39) was superior over other treatments. F_3 (1.29) was superior in potassium content in leaves. Interaction effect showed significant difference in treatments with S_3F_3 (1.46) which was superior over other treatments.

4.5.4 Calcium (%)

The effect of treatments on calcium content in leaf is given in the Table 12. Calcium content was found to be non significant with respect to different levels of seed treatment. Among the foliar spray F_3 (3.19) showed highest calcium content in leaf. Interaction effect did not show considerable difference among treatments.

Table 12: Effect of seed treatment, foliar spray and their interactions on N, P, K, Ca, Mg and S content in cowpea leaf

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Levels of seed treatment						
S_1	2.00	0.22	1.02	3.06	0.43	0.22
S_2	2.07	0.22	1.22	3.12	0.63	0.22
S_3	2.04	0.26	1.39	3.14	0.88	0.23
S_4	2.04	0.23	1.21	3.07	0.59	0.22
SEm (±)	0.02	0.002	0.01	0.02	0.01	0.002
CD (0.05)	NS	0.006	0.02	NS	NS	NS
Levels of foliar spray						
F_1	2.15	0.23	1.17	3.05	0.59	0.22
F_2	2.30	0.24	1.16	3.12	0.66	0.22
F_3	3.08	0.25	1.29	3.19	0.64	0.22
SEm (±)	0.02	0.002	0.009	0.02	0.01	0.002
CD (0.05)	0.06	0.006	0.025	0.06	NS	NS
Interactions						
S_1F_1	1.21	0.18	0.94	2.99	0.41	0.22
S_1F_2	1.51	0.23	0.98	3.10	0.41	0.23
S_1F_3	3.30	0.24	1.14	3.11	0.46	0.22
S_2F_1	2.50	0.19	1.16	3.11	0.52	0.23
S_2F_2	1.31	0.22	1.17	3.11	0.54	0.22
S_2F_3	1.72	0.24	1.34	3.15	0.82	0.22
S_3F_1	2.60	0.26	1.40	3.13	0.87	0.23
S_3F_2	3.21	0.24	1.31	3.17	1.10	0.23
S_3F_3	3.70	0.28	1.46	3.13	0.66	0.23
S_4F_1	2.31	0.22	1.20	2.99	0.57	0.22
S_4F_2	3.21	0.23	1.21	3.11	0.59	0.23
S_4F_3	3.61	0.25	1.22	3.13	0.60	0.22
SEm (±)	0.04	0.003	0.01	0.04	0.01	0.004
CD (0.05)	0.13	0.010	0.05	NS	0.04	NS

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

4.5.5 Magnesium (%)

Magnesium content in cowpea leaves are given in the Table 12. Seed treatment and levels of foliar spray was found to be non significant. Among the interactions S_3F_3 (1.10) showed considerable difference among other interaction effects and was superior over other treatments.

4.5.6 Sulphur (%)

Sulphur content in leaf is presented in the Table 12. It was found to be non significant in main treatments as well as interactions.

4.5.7 Iron (mg kg^{-1})

Iron content in leaf is given in the Table 13. Levels of seed treatment showed significant difference in iron content with S_3 (377.68) recorded the highest value. F_3 (366.03) was significantly different from other foliar sprays. S_3F_3 (390) in the interaction effect showed highest iron content which was superior over other treatments.

4.5.8 Manganese (mg kg^{-1})

Manganese content in the leaf at harvest is given in the Table 13. It showed considerable difference among the treatments with S_3 (275.77), F_3 (293.30) and S_3F_3 (287.33) were superior over other treatments.

4.5.9 Zinc (mg kg^{-1})

Effect of treatments on the zinc content of leaf is presented in the Table 13. It also showed the same trend as in manganese with S_3 (61.44), F_3 (56.83) and S_3F_3 (66.13) showed highest zinc content.

4.5.10 Copper (mg kg^{-1})

Copper content in leaf with respect to different treatments are given in the Table 13. Copper content in leaf was found to be non significant with respect to different treatments and their interactions.

Table 13: Effect of seed treatment, foliar spray and their interactions on Fe, Mn, Zn, Cu, B and Mo content in cowpea leaf

Treatment	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	Mo (mg kg^{-1})
Levels of seed treatment						
S_1	334.36	212.02	47.16	28.35	25.25	1.02
S_2	327.02	217.73	49.00	28.09	25.56	1.02
S_3	377.68	275.77	61.44	27.87	26.83	1.03
S_4	358.00	235.58	52.92	27.77	26.35	1.00
SEm (±)	2.81	1.99	0.43	0.21	0.19	0.02
CD (0.05)	8.29	5.88	1.27	NS	0.58	NS
Levels of foliar spray						
F_1	336.51	217.29	49.40	28.30	25.58	1.02
F_2	345.25	225.25	51.66	28.27	25.94	1.02
F_3	366.03	293.30	56.83	28.29	26.47	1.02
SEm (±)	2.43	1.73	0.37	0.18	0.17	0.02
CD (0.05)	7.18	5.01	1.10	NS	0.50	NS
Interactions						
S_1F_1	311.00	207.07	42.85	28.32	25.02	1.02
S_1F_2	321.00	217.00	43.92	28.52	25.22	1.02
S_1F_3	371.10	270.00	54.73	28.21	25.52	1.02
S_2F_1	323.12	211.11	46.80	27.25	25.48	1.02
S_2F_2	327.10	218.02	48.60	28.40	25.18	1.02
S_2F_3	331.06	224.01	51.62	28.64	26.02	1.02
S_3F_1	371.06	267.02	56.06	27.69	26.52	1.02
S_3F_2	372.00	273.21	62.13	27.58	26.97	1.02
S_3F_3	390.00	287.33	66.13	28.36	27.02	1.02
S_4F_1	341.01	212.03	51.90	27.15	25.32	1.02
S_4F_2	361.00	223.01	52.00	28.27	26.42	1.02
S_4F_3	372.00	271.04	54.86	28.19	27.32	1.02
SEm (±)	4.86	3.50	0.75	0.36	0.34	0.02
CD (0.05)	14.37	10.20	2.21	NS	NS	NS

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

4.5.11 Boron (mg kg^{-1})

Boron content in the leaf of cowpea at harvest is given in the Table 13. Among the seed treatments S_3 (26.83) recorded highest value of boron concentration in leaf which was on par with S_4 (26.35). F_3 (26.47) was found to be superior over other levels of foliar spray. Treatment interactions were found to be non significant with respect to boron concentration in cowpea leaves at harvest.

4.5.12 Molybdenum (mg kg^{-1})

Molybdenum content in cowpea is given in the Table 13. It did not show significant difference in seed treatment, foliar spray and interaction of main treatments.

4.6 NUTRIENT CONTENT IN GRAIN

4.6.1 Nitrogen (%)

Nitrogen content in grain is given in the Table 14. S_2 (3.71) recorded highest nitrogen in grain which was superior over other seed treatments. F_3 (3.76) in foliar spray and S_2F_3 (3.91) in interaction were found to be superior over other treatments.

4.6.2 Phosphorus (%)

Grain phosphorus content in cowpea is recorded in the Table 14. It did not show significant difference in any treatments.

4.6.3 Potassium (%)

Potassium content in grain is presented in the Table 14. S_3 (1.18) in seed treatment and F_3 (1.08) in foliar spray recorded highest potassium content. Interaction effect showed that S_3F_3 (1.25) and S_4F_3 (1.19) were found to be on par with each other.

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4.6.4 Calcium (%)

Calcium content in grain of cowpea is given in the Table 14. S_3 (0.59), F_3 (0.52) and S_3F_3 (0.67) recorded maximum calcium content in grain and was superior over other treatments.

4.6.5 Magnesium (%)

Magnesium content in cowpea grain is presented in the Table 14. Magnesium content in grain was found to be non significant in main treatments as well as interactions.

4.6.6 Sulphur (%)

Sulphur content in grain is given in the Table 14. The data were found to be non significant in all the treatments.

Table 14: Effect of seed treatment, foliar spray and their interactions on N, P, K, Ca, Mg and S content in cowpea grain

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Levels of seed treatment						
S_1	3.43	0.32	0.81	0.39	0.17	0.48
S_2	3.71	0.32	1.01	0.46	0.18	0.48
S_3	3.50	0.36	1.18	0.59	0.18	0.48
S_4	3.41	0.36	1.00	0.48	0.18	0.48
SEm (±)	0.02	0.02	0.01	0.001	0.01	0.004
CD (0.05)	0.07	NS	0.04	0.004	NS	NS
Levels of foliar spray						
F_1	3.38	0.34	0.96	0.44	0.17	0.48
F_2	3.41	0.33	0.96	0.49	0.18	0.48
F_3	3.76	0.36	1.08	0.52	0.18	0.48
SEm (±)	0.02	0.02	0.01	0.001	0.014	0.003
CD (0.05)	0.06	NS	0.03	0.003	NS	NS
Interactions						
S_1F_1	3.30	0.27	0.72	0.35	0.17	0.48
S_1F_2	3.40	0.32	0.77	0.40	0.17	0.48
S_1F_3	3.61	0.37	0.93	0.43	0.17	0.48
S_2F_1	3.30	0.35	0.94	0.45	0.18	0.48
S_2F_2	3.31	0.34	0.96	0.47	0.18	0.48
S_2F_3	3.91	0.34	1.13	0.48	0.18	0.48
S_3F_1	3.71	0.35	1.01	0.51	0.17	0.48
S_3F_2	3.62	0.37	1.10	0.61	0.17	0.48
S_3F_3	3.81	0.40	1.25	0.67	0.17	0.48
S_4F_1	3.23	0.37	0.99	0.47	0.17	0.48
S_4F_2	3.31	0.34	1.00	0.48	0.17	0.48
S_4F_3	3.72	0.38	1.19	0.51	0.17	0.48
SEm (±)	0.01	0.02	0.02	0.003	0.021	0.006
CD (0.05)	0.02	NS	0.07	0.010	NS	NS

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

4.6.7 Iron (mg kg^{-1})

Percentage of iron in grain is given in the Table 15. Seed treatment S_3 (120.91), foliar spray F_3 (117.65) and interaction S_3F_3 (131.96) were found to be superior in the respective treatments and their combinations.

4.6.8 Manganese (mg kg^{-1})

Manganese content in grain is given in the Table 15. Among the seed treatments, S_3 (14.30) recorded significantly higher value. F_3 (12.71) and S_3F_3 (17.08) showed superior value in the respective treatments and interaction.

4.6.9 Zinc (mg kg^{-1})

Zinc content in grain is given in the Table 15. It also showed the same trend of iron and manganese with S_3 (56.90), F_3 (53.98) and S_3F_3 (59.21) recorded superior values.

4.6.10 Copper (mg kg^{-1})

Copper content in grain also showed significant difference among treatments which is given in the Table 15. S_3 (6.50), F_3 (6.45) and S_3F_3 (7.02) showed copper content superior over other treatments.

4.6.11 Boron (mg kg^{-1})

Boron content in grain is given in the Table 15. S_3 (86.80), F_3 (83.42) and S_3F_3 (89.23) showed highest boron content over other treatments.

4.6.12 Molybdenum (mg kg^{-1})

Molybdenum content in grain is given in the Table 15. It was found to be non significant with respect to seed treatment, foliar spray and their interaction.

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Table 15: Effect of seed treatment, foliar spray and their interactions on Fe, Mn, Zn, Cu, B and Mo content in cowpea grain

Treatment	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	Mo (mg kg^{-1})
Levels of seed treatment						
S_1	103.54	7.74	48.63	5.23	74.80	1.05
S_2	105.86	8.96	51.13	5.53	75.11	1.06
S_3	120.91	14.30	56.90	6.50	86.80	1.07
S_4	109.50	10.74	52.82	6.06	79.13	1.06
SEm (±)	0.03	0.01	0.01	0.001	0.01	0.02
CD (0.05)	0.09	0.03	0.02	0.003	0.04	NS
Levels of foliar spray						
F_1	105.35	9.21	50.94	5.45	76.20	1.04
F_2	106.85	9.38	52.19	5.60	77.25	1.05
F_3	117.65	12.71	53.98	6.45	83.42	1.05
SEm (±)	0.02	0.01	0.009	0.001	0.01	0.02
CD (0.05)	0.07	0.03	0.025	0.002	0.04	NS
Interactions						
S_1F_1	94.83	5.55	45.06	4.50	69.50	0.77
S_1F_2	100.83	5.56	48.10	4.71	70.30	0.69
S_1F_3	114.96	12.11	52.73	6.50	84.61	1.30
S_2F_1	104.90	8.56	50.30	5.21	74.50	1.49
S_2F_2	103.86	9.06	50.90	5.41	75.50	1.54
S_2F_3	108.83	9.26	52.21	6.01	75.31	0.89
S_3F_1	112.86	12.86	55.81	6.20	84.61	1.52
S_3F_2	117.90	12.96	55.92	6.32	86.61	1.25
S_3F_3	131.96	17.08	59.21	7.02	89.23	1.56
S_4F_1	108.83	9.89	52.61	5.91	76.21	1.24
S_4F_2	104.83	9.94	53.86	6.01	76.60	1.35
S_4F_3	114.83	12.39	52.00	6.30	84.61	1.45
SEm (±)	0.05	0.02	0.01	0.002	0.02	0.03
CD (0.05)	0.15	0.06	0.05	0.004	0.08	NS

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

4.7 NUTRIENT UPTAKE IN COWPEA GRAIN

4.7.1 Nitrogen uptake (kg ha^{-1})

Nitrogen uptake in cowpea grain is given in Table 16. Among the seed treatment nitrogen uptake in grain was significantly higher in S_3 (82.75). Foliar spray, F_3 (81.87) showed highest uptake among the different levels of foliar spray. Interaction was found to be non significant with respect to nitrogen uptake in grain.

4.7.2 Phosphorus uptake (kg ha^{-1})

Phosphorus uptake in cowpea grain is given in Table 16. Phosphorus uptake in grain was significantly higher in treatments S_3 (8.29) and F_3 (7.87). Interaction S_3F_3 (8.97) showed highest uptake which was significantly different from other interactions.

4.7.3 Potassium uptake (kg ha^{-1})

Potassium uptake in cowpea grain is given in Table 16. Seed treatment S_3 (26.47) showed the highest uptake of potassium in grain which was significantly different from all other levels of seed treatment. among the foliar spray, F_3 (23.70) showed significantly higher uptake of potassium in cowpea grain. S_3F_3 (29.19) recorded highest uptake of potassium compared to other interactions and was found to be superior.

4.7.4 Calcium uptake (kg ha^{-1})

Calcium uptake in cowpea grain is given in Table 16. Among seed treatment and foliar spray calcium uptake by cowpea grain was significantly superior in S_3 (13.43) and F_3 (11.52) respectively. Considering the interaction, S_3F_3 (15.64) recorded significantly higher uptake of calcium compared to other interactions.

4.7.5 Magnesium uptake (kg ha^{-1})

Magnesium uptake in cowpea grain is given in Table 16. Levels of seed treatment showed significant difference in the uptake of magnesium by cowpea grain. S_3 (3.88) showed highest uptake which was on par with S_4 (3.78). Levels of foliar spray also showed significant difference in the uptake of magnesium. F_3

(3.75) showed highest uptake which was on par with F_2 (3.73). interaction was found to be non significant.

4.7.6 Sulphur uptake (kg ha^{-1})

Sulphur uptake in cowpea grain is given in Table 16. Among the seed treatment sulphur uptake by grain was significantly higher in S_3 (10.79) which was on par with S_4 (10.54). F_3 (10.55) showed highest uptake significantly higher and was found to be on par with F_2 (10.18). interaction effect was found to be non significant.

4.7.7 Iron uptake (kg ha^{-1})

Iron uptake in cowpea grain is given in Table 17. Iron uptake in grain was significantly higher in S_3 (0.27) compared to other seed treatments and F_3 (0.25) recorded significantly higher uptake of iron among the different levels of foliar spray. Interaction S_3F_3 (0.30) recorded highest uptake of iron in cowpea grain and was found to be significantly higher compared to other interactions.

4.7.8 Manganese uptake (kg ha^{-1})

Manganese uptake in cowpea grain is given in Table 17. Manganese uptake in grain was found to be non significant with respect to seed treatment and foliar spray. Interaction S_3F_3 (0.04) recorded highest uptake significantly different from all other interactions.

4.7.9 Zinc uptake (kg ha^{-1})

Zinc uptake in cowpea grain is given in Table 17. Zinc uptake was significantly higher in S_3 (0.12) and F_3 (0.12). Interaction S_3F_3 (0.14) showed significantly higher uptake compared to other interactions.

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4.7.10 Copper uptake (kg ha^{-1})

Copper uptake in cowpea grain is given in Table 17. Copper uptake in cowpea grain was found to be non significant with respect to seed treatment, foliar spray and their interactions.

4.7.11 Boron uptake (kg ha^{-1})

Boron uptake in cowpea grain is given in Table 17. Boron uptake was significantly higher in S_3 (0.19) and F_3 (0.18). Interaction S_3F_3 (0.21) showed significantly higher uptake compared to other interactions.

4.7.12 Molybdenum uptake (kg ha^{-1})

Molybdenum uptake in cowpea grain is given in Table 17. Molybdenum uptake in cowpea grain was found to be non significant with respect to seed treatment, foliar spray and their interactions.

Table 16: Effect of seed treatment, foliar spray and their interactions on uptake of N, P, K, Ca, Mg and S in cowpea grain

Treatment	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})	Mg (kg ha^{-1})	S (kg ha^{-1})
Levels of seed treatment						
S_1	68.20	6.48	16.14	7.87	3.41	9.57
S_2	69.21	7.13	20.05	9.28	3.56	9.49
S_3	82.75	8.29	26.47	13.43	3.88	10.79
S_4	74.29	7.90	21.85	10.64	3.78	10.54
SEm (±)	1.18	0.13	0.36	0.18	0.06	0.16
CD (0.05)	3.48	0.37	1.08	0.53	0.19	0.47
Levels of foliar spray						
F_1	67.17	6.81	19.35	8.95	3.50	9.56
F_2	71.80	7.50	20.33	10.45	3.73	10.18
F_3	81.87	7.87	23.70	11.52	3.75	10.55
SEm (±)	1.10	0.11	0.32	0.15	0.05	0.14
CD (0.05)	3.02	0.32	0.94	0.46	0.16	0.41
Interactions						
S_1F_1	60.09	4.97	13.23	6.37	3.15	8.80
S_1F_2	68.03	6.40	15.40	8.13	3.46	9.67
S_1F_3	76.48	8.05	19.77	9.11	3.60	10.24
S_2F_1	60.91	6.58	17.53	8.36	3.32	8.86
S_2F_2	66.14	7.66	19.20	9.46	3.73	9.66
S_2F_3	80.58	7.14	23.42	10.03	3.64	9.96
S_3F_1	79.24	7.62	25.65	11.04	3.77	10.33
S_3F_2	80.50	8.29	24.59	13.62	3.85	10.74
S_3F_3	88.50	8.97	29.19	15.64	4.02	11.30
S_4F_1	68.43	8.28	20.99	10.04	3.74	10.25
S_4F_2	72.53	7.59	22.13	10.58	3.87	10.66
S_4F_3	81.92	7.62	22.44	11.29	3.74	10.70
SEm (±)	2.04	0.22	0.64	0.31	1.11	0.27
CD (0.05)	NS	0.64	1.90	0.92	NS	NS

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

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Table 17: Effect of seed treatment, foliar spray and their interactions on uptake of Fe, Mn, Zn, Cu, B and Mo in cowpea grain

Treatment	Fe (kg ha^{-1})	Mn (kg ha^{-1})	Zn (kg ha^{-1})	Cu (kg ha^{-1})	B (kg ha^{-1})	Mo (kg ha^{-1})
Levels of seed treatment						
S_1	0.20	0.01	0.09	0.01	0.14	0.002
S_2	0.20	0.01	0.10	0.01	0.14	0.003
S_3	0.27	0.03	0.12	0.01	0.19	0.003
S_4	0.23	0.02	0.11	0.01	0.17	0.003
SEm (±)	0.004	-	0.002	0.003	0.003	0.00013
CD (0.05)	0.01	0.001	0.005	NS	0.008	NS
Levels of foliar spray						
F_1	0.21	0.02	0.10	0.01	0.15	0.003
F_2	0.23	0.02	0.11	0.01	0.16	0.003
F_3	0.25	0.03	0.12	0.01	0.18	0.003
SEm (±)	0.003	-	0.002	0..003	0.002	0.00012
CD (0.05)	0.01	0.001	0.005	NS	0.007	NS
Interactions						
S_1F_1	0.17	0.01	0.08	0.008	0.12	0.001
S_1F_2	0.20	0.01	0.09	0.009	0.14	0.001
S_1F_3	0.24	0.03	0.11	0.14	0.17	0.003
S_2F_1	0.19	0.02	0.09	0.01	0.13	0.003
S_2F_2	0.20	0.02	0.10	0.01	0.15	0.003
S_2F_3	0.22	0.02	0.11	0.01	0.15	0.002
S_3F_1	0.24	0.03	0.12	0.01	0.18	0.003
S_3F_2	0.26	0.03	0.12	0.01	0.19	0.003
S_3F_3	0.30	0.04	0.14	0.01	0.20	0.004
S_4F_1	0.23	0.02	0.11	0.01	0.16	0.003
S_4F_2	0.23	0.02	0.12	0.01	0.16	0.003
S_4F_3	0.25	0.03	0.11	0.01	0.18	0.003
SEm (±)	0.007	0.001	0.003	0.003	0.005	0.00013
CD (0.05)	0.02	0.002	0.009	NS	0.014	NS

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

4.7 SOIL BIOMASS CARBON (mg kg^{-1} soil)

The data recorded on soil biomass carbon in soil is given in the Table 16. It was found to be non significant with respect to treatments and interactions.

Table 18: Effect of seed treatment, foliar spray and their interactions on soil biomass carbon content

Treatment	Soil biomass carbon (mg kg^{-1} soil)
Levels of seed treatment	
S_1	168.14
S_2	167.18
S_3	169.57
S_4	168.17
SEm (±)	1.271
CD (0.05)	NS
Levels of foliar spray	
F_1	167.32
F_2	168.61
F_3	168.86
SEm (±)	1.101
CD (0.05)	NS
Interactions	
S_1F_1	168.45
S_1F_2	167.98
S_1F_3	168.00
S_2F_1	166.87
S_2F_2	167.70
S_2F_3	166.98
S_3F_1	168.32
S_3F_2	170.34
S_3F_3	170.06
S_4F_1	165.65
S_4F_2	168.45
S_4F_3	170.42
SEm (±)	2.202
CD (0.05)	NS

S_1- no seed treatment; S_2- seed treatment with rhizobium; S_3- seed treatment with micronutrient formulation (best from Exp. 1); S_4- seed treatment with micronutrient formulation (second best from Exp. 1)

F_1- no foliar spray; F_2- one foliar application at 15 DAS; F_3- two foliar application at 15 & 30DAS

Discussion

5. DISCUSSION

The results obtained from the study "Evaluation of micronutrient formulation on cowpea (*Vigna unguiculata* L. Walp)" are briefly discussed in this chapter. The investigation was conducted in three different steps which includes standardization of micronutrient mixture for cowpea, seed treatment and field experiment.

5.1 STANDARDIZATION OF MICRONUTRIENT FORMULATION FOR COWPEA

A micronutrient formulation developed at college of agriculture, Padannakad has been modified for the foliar application and seed treatment of cowpea. The formulation is a mixture of two solutions, Solution A and Solution B. Solution A contains iron sulphate, manganous sulphate, zinc sulphate, copper sulphate, boric acid and ammonium molybdate at definite proportion. Solution B is an organically chelated solution. Solution A and solution B should be mixed together before foliar spray and after mixing both the solutions the formulation cannot be stored due to its poor shelf life and precipitate formation. Several trials were done to reduce precipitate formation of this formulation when mixed together. A specific combination of solutions A and B which do not form precipitate and could be stored as such for longer duration was formulated after several trials. Thus it could help the farmers to reduce the labour of mixing both solutions A and solution B during foliar spray. The formulation would definitely help in boosting the production and productivity of pulses.

5.2 SEED TREATMENT STUDY

5.2.1 Standardization of time and dose of micronutrient formulation for seed treatment of cowpea

The different concentrations of micronutrient formulation and time required for seed treatment in cowpea was standardized. Cowpea seeds were treated with micronutrient mixture at different concentrations (0.25 %, 0.50 %, 0.75 %, 1 %, 1.5 % and 2 %) for different durations (1 hr, 2hrs, 4 hrs, 6hrs, 8 hrs, 10 hrs and 12 hrs).

Observations on seed germination and its emergence showed that the seeds treated with micronutrient mixture for 6 hours showed better establishment for the different concentration of micronutrient formulation as compared to other duration of seed treatment. Time for seed treatment was standardized in papaya for boron. Seeds treated with 2 ppm boron solution for six hours showed increased germination and growth of seedlings (Deb *et al.*, 2010).

5.2.2 Effect of seed treatment on seedling characters of cowpea

Cowpea seeds were treated with micronutrient mixture at 0.25 %, 0.50 %, 0.75 %, 1 %, 1.5 % and 2 % for six hours. The biometric observations obtained from seed treatment study are briefly discussed below.

The different concentrations of micronutrient mixture in seed treatment study had no significant influence on germination percentage and days taken for germination whereas seedling length at three leaf stage and seedling vigour index was significantly influenced by the treatments. The treatment T_6 (micronutrient mixture @ 2 %) showed maximum seedling length at three leaf stage and seedling vigour index which might be due to the beneficial effect of micronutrients in enhancing cell elongation and cell division of meristematic tissues. The increased seedling length of cowpea contributed to considerable increase in seedling vigour index in the treatment receiving 2 % concentration of micronutrient mixture. Thus T_6 was considered as the best concentration for seed treatment study. This treatment was followed by T_5 (micronutrient mixture @ 1.5 %) which recorded the second highest values in seedling length at three leaf stage and seedling vigour index. The treatments T_6 (best seed treatment) and T_5 (second best) were selected for the field experiment to evaluate the performance of micronutrient formulation in cowpea. Masuthi *et al.* 2009 had reported that cowpea seeds treated with zinc sulphate showed higher vigour index and Deb *et al.* 2010 reported that papaya seeds treated with boron at 2ppm showed better growth of seedlings.

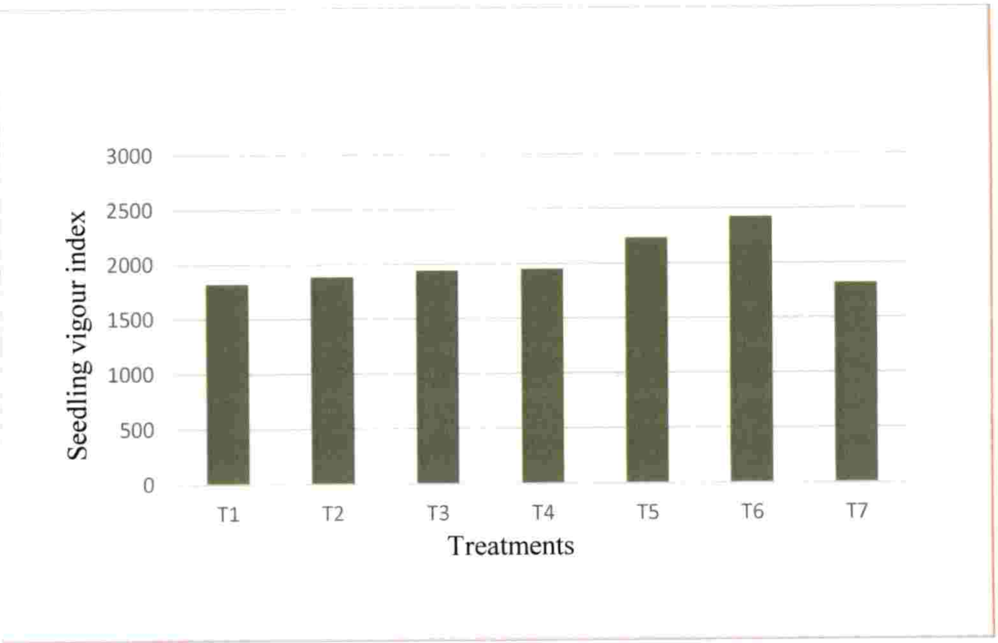

Fig. 1 Effect of seed treatment on seedling vigour index

5.3 FIELD EXPERIMENT

5.3.1 Effect of seed treatment, foliar spray of micronutrient formulation and their interaction on growth attributes

The results obtained from the study revealed that growth attributes like plant height and stem diameter at flower bud initiation and harvest were influenced by seed treatment, foliar spray of micronutrient formulation and their interactions.

Considering the effect of seed treatment, it was evident that plants subjected to micronutrient seed treatment at 2 percentage (S_3) and 1.5 percentage (S_4) showed remarkable variation in plant height at flower bud initiation stage. At harvest seeds treated with 2 percentage micronutrient formulation showed maximum plant height compared to other treatments. With respect to foliar application of micronutrient formulation plants supplied with micronutrient mixture (2 percentage formulation was used for the foliar spray) at 15 DAS and 30 DAS (F_3) showed characteristic difference in plant height compared to single foliar spray as well as no foliar spray at both flower bud initiation stage and harvest. Among the interactions plants receiving 2 per cent seed treatment along with two foliar spray (S_3F_3) showed maximum plant height at both the stages. Hence the application of micronutrient mixture as seed treatment and foliar spray was found to be the best treatment. The beneficial effect of micronutrients in growth and development of plants might be the reason for increase in plant height compared to control treatment receiving no seed treatment and no foliar spray. Micronutrients has the capacity to increase the auxin content especially zinc and chlorophyll content of tissues thus promoting plant growth and yield. It helps in the growth of cells by promoting cell elongation and cell division of meristematic regions. The micronutrients zinc and boron plays a crucial role in growth and development of new cells in plants. The findings are in accordance with the results obtained by Suryanarayana and Reddy (1978). They reported that plant height of French bean was increased by the application of 0.01 per cent Zn and B. Cowpea seeds treated with borax showed highest plant height at 30 and 60 days after sowing (Masuthi *et al*., 2009). Application of micronutrients enhanced the plant height of tomato over the control by 30 per cent (Ejaz *et al*. (2011).

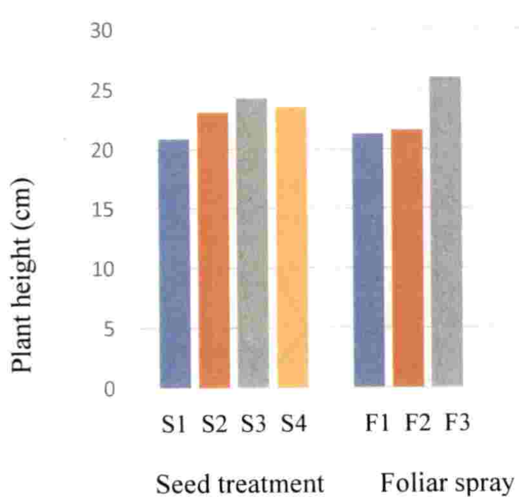

Fig.2 Effect of micronutrient formulation on plant height at flower bud initiation stage

In the case of stem diameter, effect of seed treatment with micronutrient (S_3 and S_4) and rhizobium (S_2) was significantly evident at flower bud initiation stage. The beneficial effect of micronutrients and rhizobium on growth and development of plants were clearly understood from these observations. Micronutrients helps in plant growth by cell elongation and division. Rhizobium also increases the growth of plants. It stimulates the growth of plants by production of plant hormones. But at the time of harvest seed treatment with micronutrient mixture at 2 percentage showed the highest stem diameter compared to seeds treated with rhizobium, 1.5 percentage micronutrient and no seed treatment. Thus micronutrient enhanced the growth of plants. In treatments receiving foliar spray of micronutrient mixture, at flower bud initiation stage both F_2 and F_3 (one foliar spray and two foliar spray) showed highest stem diameter and at harvest the plants receiving two foliar spray showed highest stem diameter. From this it is evident that treatments receiving two foliar spray showed maximum stem diameter. The beneficial effect of micronutrient spray on cowpea could be concluded from the data. The interaction effect showed that at flower bud initiation stage S_2F_3 (seed treatment with rhizobium + two foliar spray) showed maximum stem diameter which was on par with S_3F_2 (seed treatment with 2 % micronutrient mixture + one foliar spray), S_4F_3 (seed treatment with 1.5 % micronutrient mixture + two foliar spray) and S_4F_2 (seed treatment with 1.5 % micronutrient mixture + one foliar spray). While at harvest treatment receiving rhizobium and two foliar spray showed highest stem diameter. Thus showing the beneficial effect of both micronutrients and rhizobium in plant growth and development. These findings were in accordance with Mathur (2000) reported that seeds of garden pea inoculated with rhizobium showed enhanced growth characters and yield. Suryanarayana and Rao (1981) reported that foliar spray of micronutrient solution containing zinc, copper, manganese, magnesium, boron and molybdenum resulted in enhanced growth of okra. Bukvic *et al.* (2003) reported the similar increasing trend in stem diameter with zinc application in maize.

Dry matter production also showed considerable variations with respect to different treatments.

Considering the levels of seed treatment dry matter production was found to be highest in seed treatment with micronutrient formulation at two percentage (S_3). It was found to be superior over other seed treatments. In foliar spray dry matter production was highest in plants receiving two foliar spray (F_3) which was also superior compared to other levels of foliar sprays. Hence in the interaction effect S_3F_3 showed considerably higher production of dry matter compared to other interactions. Seed treatment with micronutrients have the capacity to enhance dry matter production. Seed treatment might had resulted in increased seed vigour. Increased seed vigour might be the reason for maximum dry matter production in plants. Similar results were obtained in barley. Seeds of barley treated with micronutrients showed increased dry matter production (Ajouri *et al.*, 2004).

5.3.2 Effect of seed treatment, foliar spray of micronutrient formulation and their interaction on nodulation of cowpea

The results obtained from the study revealed that seed treatment with rhizobium has a positive effect on the number of nodules whereas seed treatment and foliar spray of micronutrient formulation did not result in considerable increase in the number of nodules. The fresh weight and dry weight of nodules were also found to be non significant in seed treatment, foliar spray and their interaction. Rhizobium forms symbiotic association with the roots of legumes and helps fixing nitrogen in the soil. Cowpea, a legume crop has the inherent capacity to fix nitrogen by the formation of root nodules. Inoculation of cowpea seeds with specific rhizobium strains results in increased nodule formation and thus increases the nitrogen fixation in soil. Rhizobia can infect the legume hosts and induce nodule formation. This might be the reason for increased number of nodules in plants inoculated with rhizobium bacteria. Similar results were obtained in cowpea seeds inoculated with rhizobium showed increased nodulation compared to non inoculated seeds (Pant *et al.*, 2000). Yadav (2000) reported that cowpea plants treated with vermicompost and rhizobium showed more number of nodules compared to the control.

5.3.3 Effect of seed treatment, foliar spray of micronutrient formulation and their interaction on yield and yield attributes of cowpea

There was no considerable effect of treatment in the number of days taken for first flowering, number of seeds per pod and length of pod. However, number of pods per plant and pod weight per plant was found to be influenced by the treatments.

Considering the levels of seed treatment, number of pods per plant and pod weight per plant was found to be highest in seed treatment with micronutrient formulation at two percentage (S_3) and was on par with S_4 (seed treatment with 1.5 % micronutrient formulation). While considering foliar spray, number and weight of pods per plant were found to be highest in treatments receiving two foliar spray (F_3). Hence the interaction effect of these three traits followed the same trend with highest values recorded in S_3F_3 (seeds treated with micronutrient formulation at two percentage + two foliar spray) followed by S_4F_3 (seeds treated with micronutrient formulation at 1.5 percentage + two foliar spray). Considering the grain yield of cowpea, highest yield was obtained in S_3, F_3 and S_3F_3 indicating the essentiality of micronutrients in increasing the yield of cowpea. The role of micronutrients in enhancing the yield and its attributes are evident. Seed treatment with micronutrients had positive influence on yield of cowpea. Seed treatment with micronutrients might had increased the seed vigour resulting in better growth and yield of plants. Micronutrients are involved in active photosynthesis and activates several enzymes by increasing the catalytic activity. It is also involved in hormonal metabolism, pollination and fertilization and enhancement of cell division which ultimately leads to increase in number and weight of pods. By enhancing the cell division and expansion vegetative growth can also be increased. It also helps in increased production of assimilates and its proper partitioning. These findings are in accordance with the results reported by Mallick and Muthukrishnan (1980). Tomato plants treated with micronutrient foliar spray showed increased fruit weight. Pranakrishna (1976) reported that pulse crops treated with sodium molybdate increased the seed yield by 45 percentage compared to the control.

Common beans treated with zinc increased the yield of crops (Kaya *et al*., 2007). Pod yield in pea was found to be higher in seeds treated with 0.5 per cent boron solution (Kumar *et al*., 2008). Foliar application of micronutrient mixture (zinc, copper, boron and iron) at two percentage resulted in increased yield in okra by 19 per cent (Datire *et al*., 2010).

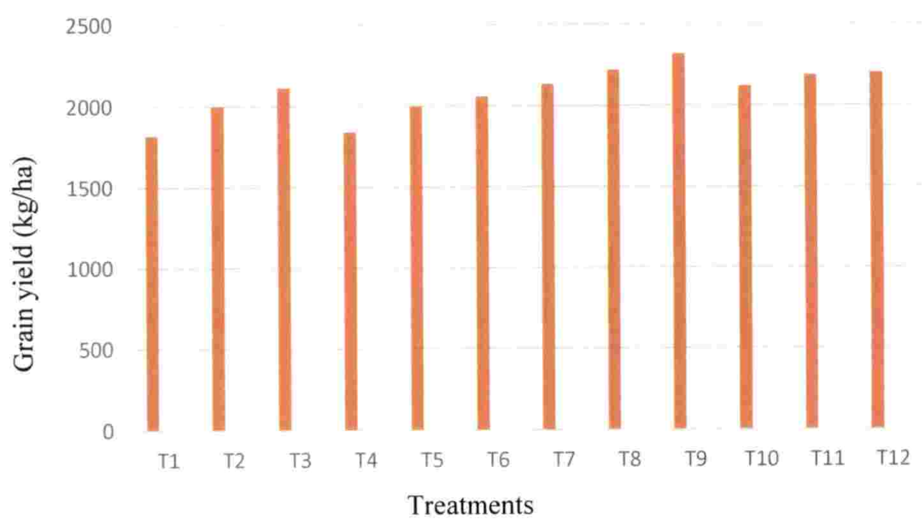

Fig. 3 Effect of treatments on grain yield of cowpea

5.3.4 Effect of seed treatment, foliar spray of micronutrient formulation and their interaction on root cation exchange capacity (root CEC)

Seed treatment and foliar spray of micronutrient formulation showed considerable influence in the cation exchange capacity of roots. Seeds treated with micronutrient mixture at 2 percentage showed higher root CEC. Double spray of micronutrient formulation showed higher root CEC compared to single spray and no foliar spray.

Interaction effect showed higher root CEC in S_3F_3 (seeds treated with micronutrient formulation at two percentage + two foliar spray) which was on par with S_2F_3 (seed treatment with rhizobium + two foliar spray). Root cation exchange capacity is defined as the capacity of the root to exchange the cations with the soil cations. As root CEC increases the uptake of nutrients also increases which results in better growth and development. Seeds treated with micronutrient solution showed increased seed vigour and this might be the reason for better seedling growth and emergence resulting in shoot and root development. Enhanced growth of plants could have influenced the uptake of nutrients from soil by increasing the exchange of cations between root surface and soil. Rhizobium also enhances the nutrient uptake of plants, which might be the reason for higher root CEC in seeds treated with rhizobium. This results goes in line with the findings of Pattanayak *et al.* 2000. He observed that green gram seeds treated with sodium molybdate along with rhizobium showed increased nutrient uptake, plant growth and yield of crops. El-Fouly and EL-Sayed in 1997 reported that foliar spray of micronutrients increases the uptake of nutrients through soil by promoting root growth.

5.3.5 Effect of seed treatment, foliar spray of micronutrient formulation and their interaction on soil nutrient status

The effect of treatments and their interaction on the soil nutrient status is briefly discussed below.

5.3.5.1 Soil pH and EC

Soil pH and electrical conductivity were found to be non significant with respect to different treatments and their interaction. The plots treated with micronutrient solution and double foliar spray showed the lowest pH values compared to other plots. Also seeds treated with rhizobium and single foliar spray exhibited lowest pH in the interaction at 45 days after sowing and harvest. Electrical conductivity was found to be highest in seed treatment with two percentage micronutrient mixture, double foliar spray and their interaction at both 45 days after sowing and at harvest. Electrical conductivity of the soil solution was the controlled sum of cation and anion concentrations (Lipman *et al.*, 1926).

5.3.5.2 Organic carbon

Organic carbon content in the soil was found to be non significant at 45 days after sowing and harvest in treatments receiving different levels of seed treatment. Treatments receiving micronutrient foliar spray at 15 days after sowing and 30 days after sowing showed increased organic carbon content in the soil at the time of harvest. While organic carbon at 45 days after sowing was found to be non significant. Interaction effect was found to be non significant at 45 days after sowing. However, at harvest S_3F_3 also showed highest organic carbon content which was on par with seed treatment with rhizobium + two foliar spray (S_2F_3), seed treatment with 1.5 percentage micronutrient formulation + one foliar spray, two foliar spray and no foliar spray (S_4F_2, S_4F_3 and S_4F_1) and seed treatment with 2 percentage micronutrient formulation + one foliar spray (S_3F_2). Foliar spray of micronutrient formulation increased the organic carbon content in soil (Premalatha, 2016).

5.3.5.3 Available nitrogen

Available nitrogen in the soil was highest in the soil where cowpea seeds treated with rhizobium were planted (S_2) at both 45 days after sowing and harvest. Levels of foliar spray and the interaction effect was non significant in the amount of nitrogen available in the soil at both stages. Nitrogen content of soils treated with rhizobium showed higher nitrogen content compared to others. The enhanced nitrogen fixation of legumes in symbiotic association with rhizobium might be the reason for increase in the nitrogen content of soil at the time of harvest. Similar results were reported by Carsky *et al.* 2002.

5.3.5.4 Available phosphorus

Available phosphorus content in the soil was found to be the highest in treatment S_2 (seeds treated with rhizobium) at both 45 days after sowing and harvest. The reason for this might be the inoculation of rhizobium. Rhizobium has the capacity to solubilize the phosphorus present in the unavailable form to available form and make it available for plants. Nyoki and Ndakidemi (2018) reported that soybean seeds treated with rhizobium showed increased uptake of phosphorus. Available phosphorus was found to be non significant at 45 days after sowing and at harvest for both foliar spray and interactions.

5.3.5.5 Available potassium

Available potassium in the soil was found to be highest in treatment S_3 (seed treatment with 2 percentage micronutrient mixture), F_3 (double foliar spray of micronutrient mixture) and S_3F_3 (micronutrient seed treatment at two percentage and double foliar spray) at both 45 DAS and harvest. From this it is evident that the micronutrients have capacity to increase the availability of potassium for plants. This fall in line with the findings of Sekhon and Singh (2013). They concluded that availability of potassium for the crop can be enhanced by the application of micronutrients along with macronutrients. Enhanced availability of potassium may be due to the fact that micronutrients ensure the efficient use of macronutrients (Swati *et al.*, 2011).

5.3.5.6 Available micronutrients (Fe, Mn, Zn, Cu, B and Mo)

Different seed treatments did not show any significant difference in the status of these micronutrients in soil at 45 days after sowing except in the case of boron. Boron was found to be superior in S_3 (seed treatment with micronutrient mixture at 2 %). Significant difference in the concentration of iron, manganese, zinc and molybdenum in soil were recorded at harvest. Content of iron, zinc and manganese in soil was superior in S_3 which was on par with S_4 (seed treatment with micronutrient mixture at 2% and 1.5% respectively). Molybdenum content at harvest recorded highest value in S_3. This indicates that seed treatment had pronounced effect on micronutrient concentration in soil. Pattanayak *et al.* 2000; Nyoki and Ndakidemi, 2018 reported similar results.

Considering different levels of foliar spray, F_3 (double foliar spray of micronutrient mixture) recorded the highest status of iron, manganese, zinc, boron and copper at 45 days after sowing. At harvest iron, manganese and copper were found to be superior over other treatments and zinc content was highest in F_3 which was on par with F_2 (single foliar spray). Boron content was found to be non significant at harvest stage. Molybdenum was significant only at harvest receiving treatment F_3. Thus foliar spray of micronutrient mixture provided a significant role in increasing the micronutrient concentration in soil also. Similar results were reported by Alva (2009), Thiyageshwari and Ramanathan (2001), Ravi *et al.* (2008).

Treatment interactions showed significant changes in the availability of micronutrients. Manganese, zinc, copper and boron were found to be higher in treatments receiving both seed treatment and two foliar spray of micronutrient mixture (S_3F_3) at 45 days after sowing. Iron showed significantly higher values in S_1F_3 (no seed treatment and double foliar spray). At harvest iron and manganese status were found to be higher in treatment receiving both micronutrient seed treatment (S_3) and two foliar spray. Zinc, copper, boron and molybdenum were non significant with respect to treatment interactions at harvest.

5.3.6 Effect of seed treatment, foliar spray of micronutrient formulation and their interaction on nutrient content of cowpea leaves

5.3.6.1 Nitrogen content in leaf

Nitrogen content in the leaf was found to be non significant with respect to different levels of seed treatment. Among the foliar spray, treatments receiving two foliar spray of micronutrient mixture at 15 days after sowing and 30 days after sowing showed considerably higher nitrogen content in leaves compared to other levels of foliar spray. In treatment interactions, treatments receiving micronutrient seed treatment along with two foliar spray of micronutrient mixture showed highest nitrogen content in cowpea leaves while the lowest concentration was obtained in control. The essentiality of micronutrients in plant is evident in the result of the study. Similar results were obtained by Premalatha (2016) in banana and Ashwini (2018) in okra.

5.3.6.2 Phosphorus content in leaf

Phosphorus content in leaf showed considerable difference with respect to treatments and their interactions. Seed treatment with 2 % micronutrient mixture showed highest phosphorus content in cowpea leaves. Among foliar spray phosphorus was found to be highest in two foliar sprays of micronutrient mixture. In the interaction effect combination of these two treatments (seed treatment and foliar spray of micronutrient mixture) showed highest value which was significantly different from other interactions. Hence it is evident that micronutrients help in the uptake of phosphorus by plants. These nutrients also promote phosphate metabolism and translocation to different parts of plants. Similar results were reported by Ashwini (2018). She reported an increase in the phosphorus content of bhindi leaves by foliar application of micronutrient mixture.

5.3.6.3 Potassium content in leaf

Average potassium concentration in cowpea leaves followed the similar trend as in leaf phosphorus content. Potassium concentration was found to be highest in both seed treatment and double foliar spray of micronutrient mixture as well as their interaction. Thus the beneficial effect of micronutrients in enhancing potassium concentration in leaves of cowpea is revealed in this study. The presence of boron in the micronutrient formulation might be the reason for the enhanced potassium uptake in plants. Similar results were obtained by Ashwini (2018) and Premalatha (2016).

5.3.6.4 Calcium, magnesium and sulphur concentration in leaf

Among the secondary nutrients, calcium was found to be significant only with the application of micronutrient mixture as foliar spray. Magnesium was showed considerable difference among the interaction effects and was found to be superior in treatment receiving micronutrient seed treatment (S_3) and two sprays of micronutrient formulation. Sulphur was non significant among the treatments. Premalatha (2016) reported an increase in calcium content of banana leaves sprayed with 3 % micronutrient formulation.

5.3.6.5 Micronutrient concentration in leaf at harvest

The concentration of iron, manganese, zinc and boron in the leaf were influenced by different seed treatments, levels of foliar sprays and their interactions. Fe, Mn and Zn were recorded maximum in seed treatment receiving 2 % micronutrient formulation (S_3). While B was recorded maximum in S_3 and was on par with S_4 (seed treatment with 1.5 % micronutrient formulation). Similar results were reported by Pattanayak *et al.*, 2000.

Iron, manganese, zinc and boron concentration of cowpea leaf was significantly influenced by the application of micronutrient mixture at 15 days after sowing and harvest. Similar results were recorded by Premalatha (2016) in tissue culture banana and Ashwini (2018) in bhindi. Application of zinc sulphate along with borax was found to increase the nutrient concentration in plants (Ejaz *et al.*, 2011). Foliar application of 0.5 % borax in potato at 35 days after sowing increased the nutrient concentration in plants (Das, 1977).

Iron, manganese and zinc concentration was found to be significant in the interaction S_3F_3 (seed treatment receiving 2 % micronutrient formulation + two foliar sprays). The application of micronutrients as seed treatment and foliar spray resulted in increased concentrations of these nutrients in the leaves.

5.3.7 Effect of seed treatment, foliar spray of micronutrient formulation and their interaction on nutrient content of cowpea grain

5.3.7.1 Nitrogen, phosphorus, potassium concentration and uptake in grain

Seed treatment of cowpea showed significant difference in the concentration of nitrogen and potassium in grain while phosphorus concentration was found to be non significant. Nitrogen content in grain was maximum in S_2 (seed treatment with rhizobium). This might be due to the positive effect of rhizobia on increasing the nitrogen content in grain. Thilakaratna *et al.* (2019) reported that in common bean, seed treatment with rhizobium showed increased nitrogen content in grain. Considering the potassium concentration in grain, seed treatment with micronutrient mixture at 2 % showed highest concentration. The presence of boron in the micronutrient mixture might be the reason for increase in potassium content of grain as it enhances the potassium uptake by plants.

Among the foliar spray nitrogen and potassium concentration were found to be highest in treatments receiving two foliar sprays of micronutrient mixture. Thus application of micronutrient spray could be beneficial in increasing the grain concentration of nitrogen and potassium. Since it increases the uptake of nutrients, the concentration of nutrients in plant tissues might be increased. Gad (2012) reported that application of molybdenum increased the concentration of nitrogen and potassium in groundnut seeds.

Interaction effect of treatments also showed significant difference in the concentration of nitrogen and potassium in grain. Nitrogen concentration was found to be highest in S_2F_3 (seed treatment with rhizobium + two foliar sprays). Seed treatment with rhizobium might be the reason for increase in nitrogen content which is enhanced by the foliar spray of micronutrient mixture. Potassium content in grain was found to be highest in S_3F_3 which was on par with S_4F_3. Micronutrients, especially boron help in the uptake and translocation of potassium in plants. This might be the reason for increased potassium content in grains supplied with micronutrient seed treatment and foliar spray. Pattanayak *et al.* 2000, Thiyageswari and Ramanathan, 2001 and Hanwate *et al.*, 2018 reported similar results.

Nitrogen, phosphorus and potassium uptake by grains of cowpea showed significant difference among different treatments and their interactions. The uptake of these nutrients were found to be higher in treatments receiving seed treatment with 2% micronutrient and two foliar sprays. This indicates the essentiality of micronutrients in nutrient uptake of grains. Seed treatment with micronutrients increased the uptake of nutrients (Pattanayak *et al.*, 2000). Foliar spray of micronutrients increases the uptake of nitrogen, phosphorus and potassium (Choudhary *et al.*, 2017). In the interaction effect phosphorus and potassium uptake were found to be highest in treatment receiving the best micronutrient seed treatment (S_3) and two foliar sprays. The interaction effect of this two main treatments resulted in highest nutrient uptake.

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5.3.7.2 Calcium, magnesium, sulphur concentration and uptake in grain

Treatments and their interaction showed significant difference in the concentration of calcium in cowpea grain, while magnesium and sulphur concentration were found to be non significant in main treatments and their interactions. Calcium content in grain was higher in seeds receiving micronutrient treatment (2 %) and two foliar sprays. In the interaction effect treatment S_3F_3 resulted in higher calcium content. Foliar application of micronutrients increased the nutrient concentration in potato (Das, 1977).

Considering the nutrient uptake, calcium uptake was found to be higher in seed treatment receiving 2 % micronutrient mixture and two foliar sprays and their interactions. Magnesium and sulphur uptake were higher in S_3 which was on par with S_4 (seed treatment with 1.5 % micronutrient formulation). Foliar spray F_3 recorded maximum uptake of magnesium and sulphur in grains which was on par with F_2 (single foliar spray). Interaction was found to be non significant regarding uptake of magnesium and sulphur.

5.3.7.3 Micronutrient concentration and uptake in grain

Seed treatment showed significant difference in the concentration of iron, manganese, zinc, copper and boron content in grain and were found to be highest in seeds receiving 2 % micronutrient formulation. Hence it is revealed that micronutrient seed treatment has the capacity to increase the nutrient concentration in grain. Similar results were obtained by Pattanayak *et al.*, 2000. Maize seeds treated with micronutrient increased the concentration of iron, manganese and zinc content in grains (Harris *et al.*, 2007).

Foliar application of micronutrient mixture also showed significant difference in the amount of iron, manganese, zinc, copper and boron concentration in grain. Treatments receiving foliar spray of micronutrient mixture at 15 and 30 days after sowing showed higher concentration of these micronutrients. Divyasree, 2018 reported that foliar application of micronutrient mixture containing Fe, Mn, Zn, Cu and B significantly increased the respective nutrient concentrations in grains of mung bean.

Similar results were obtained in the interaction effects. Seed treatment with the best micronutrient formulation and two foliar spray recorded highest value of micronutrients in grain except in the case of molybdenum. The combined effect of the individual treatments might be the reason for this.

Considering the trends of nutrient uptake, uptake of iron, zinc and boron was found to be highest in seed treatment receiving 2 % micronutrient mixture, two foliar sprays and their interactions. Uptake of manganese was found to be highest in the interaction receiving seed treatment with 2 % micronutrient formulation and two foliar spray. Thus micronutrient application resulted in better uptake of these nutrients in the grains of cowpea. These findings are supported by the results obtained by Pattanayak *et al.*, 2000 and Ravi *et al.*, 2008. Hanwante *et al.* 2018 reported that application of micronutrients as seed treatment along with foliar spray increased the uptake of micronutrients in soybean.

5.3.8 Incidence of pest and disease

There was no severe incidence of pest and disease observed in the field. Crops were infested with thrips during initial growth period and was controlled by the application of nimbicidine. A few plants were infected with collar rot which was controlled by drenching of copper oxychloride.

Summary

6. SUMMARY

The salient findings of the study entitled "Evaluation of micronutrient formulation in cowpea (*Vigna unguiculata* L. Walp)" are summerized in this chapter.

The investigation was carried out at College of Agriculture, Padannakkad and Regional Agricultural Research Station (RARS), Pilicode during 2017 to 2019. The objectives of the study were to evaluate the effect of micronutrient formulation through seed treatment and foliar nutrition on growth, nodulation and yield of cowpea (*Vigna unguiculata* L. Walp) and to study its effect on plant nutrient uptake and residual soil nutrient status. The study was conducted in three parts - Standardization of micronutrient formulation for cowpea, study of time and dose of micronutrient formulation for seed treatment in cowpea and field experiment to evaluate the performance of micronutrient formulation in cowpea.

Micronutrient formulation was standardized by two research workers earlier. Micronutrient formulation is a mixture of two solutions *viz*. solution A and solution B. Solution A is a mixture of zinc sulphate, boric acid, copper sulphate, manganous sulphate, ferrous sulphate and ammonium molybdate whereas solution B is an organic acid which act as a chelate. These two solutions should be mixed in a specific ratio at the time of application because of the poor shelf life of micronutrient formulation. A combination of Solution A and Solution B were prepared so that it can be supplied to farmers as micronutrient formulation which reduces the difficulty of mixing solutions during field application. The micronutrient formulation can be diluted to different concentrations for seed treatment and foliar spray.

Laboratory study was carried out at COA, Padannakkad with different concentrations of micronutrient formulations. The objective of the study was to standardize the time and dose for seed treatment in cowpea using micronutrient formulation. The experiment was carried out in completely randomized design with 7 treatments and 3 replications which included seed treatment with micronutrient formulation @ 0.25, 0.50, 0.75, 1, 1.5, 2 % as T_1, T_2, T_3, T_4, T_5 and T_6 respectively. T_7 (seed treatment with water) was the control. Seeds treated with different concentrations of micronutrient formulation were kept in petri plates for germination studies which was then planted in pots and observations were recorded upto three leaf stage. Observations on germination percentage, number of days taken for germination, seedling length at three leaf stage and seedling vigour index were recorded. Treatment T_6 (micronutrient formulation @ 2 %) showed highest seedling length at three leaf stage and seedling vigour index which was followed by treatment T_5 (micronutrient formulation @ 1.5 %). Thus the best and second best treatment from seed treatment study was concluded as T_6 and T_5 respectively.

The field experiment was carried out at Regional Agricultural Research Station (RARS), Pilicode to evaluate the effect of micronutrient formulation on cowpea. The experiment was conducted in randomized block design with 12 treatments replicated three times. The treatment consisting of combination of four levels of seed treatment (no seed treatment, seed treatment with rhizobium, seed treatment with best concentration from experiment 1 and second best seed treatment from experiment 1) and three levels of foliar application of micronutrient (no foliar application, one foliar application at 15 DAS and two foliar applications at 15 and 30 DAS). Manures and fertilizers application and other cultural practices were followed as per POP, KAU (2016) for all the treatments uniformly.

The salient findings from field experiment are summarized below

1. Different levels of seed treatment showed significant improvement on growth characters like plant height and stem diameter at flower bud initiation and harvest. Seed treatment with micronutrient formulation at 2 % (S_3) and 1.5 % (S_4) showed maximum plant height at flower bud initiation. At harvest S_3 showed highest plant height compared to others. Stem diameter was found to be higher in S_3, S_4 and S_2 (seed treatment with rhizobium) at flower bud initiation stage. At harvest S_3 recorded the

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maximum stem diameter. Whereas number of branches was non significant with respect to seed treatment.

2. Levels of foliar spray showed that foliar spray of micronutrient formulation at 15 DAS and 30 DAS (F_3) produced maximum plant height at flower bud initiation stage and harvest. Stem diameter was found to be highest in treatments receiving F_3 and F_2 (foliar spray at 15 DAS) during flower bud initiation stage while at harvest F_3 recorded the highest value.
3. Interaction effect of treatments showed that plants receiving 2 percent seed treatment along with two foliar spray (S_3F_3) produced maximum plant height at both the stages. Seed treatment with rhizobium + two foliar spray (S_2F_3) recoded maximum stem diameter at flower bud initiation stage and harvest.
4. Dry matter production was found to be highest in plants receiving seed treatment with micronutrient formulation at two percentage (S_3) among the seed treatments. Foliar spray with micronutrient formulation at 15 DAS and 30 DAS produced maximum dry matter among the different levels of foliar spray. Plants receiving seed treatment with 2 percent micronutrient formulation along with two foliar spray produced highest dry matter in the interactions compared to control.
5. Seed treatment with rhizobium produced more number of nodules per plant compared to other seed treatments. Effect of foliar spray and interaction was not significant with respect to number of nodules per plant. Also fresh weight and dry weight of nodules were not affected by different treatments.
6. Among the seed treatment S_3 (seed treatment with micronutrient formulation at two percentage) produced maximum number of pods per plant, pod weight per plant and grain yield. Similarly, treatments receiving two foliar spray and seeds treated with micronutrient formulation at two percentage + two foliar spray showed maximum yield.
7. Seed treatment and foliar spray of micronutrient formulation showed considerable influence in the cation exchange capacity of roots. Seeds treated with micronutrient formulation at 2 percentage showed higher root

CEC. Double spray of micronutrient formulation showed higher root CEC compared to single spray and no foliar spray. In the interaction effect seeds treated with micronutrient formulation at two percentage + two foliar spray recorded highest CEC which was on par with rhizobium + two foliar spray.

8. The effects of treatment application on soil nutrient status were studied at 45 DAS and at harvest stage. The pH and EC were found to be no significant. While organic carbon was found to be superior in F_3 and interaction S_3F_3 at harvest.
9. Treatments significantly influenced the available nitrogen, phosphorus and potassium in soil at 45 DAS and at harvest. S_2 showed highest available nitrogen and phosphorus at both stages. Available potassium was found to be highest in S_3, F_3 and S_3F_3 at both the stages.
10. Treatments significantly influenced the micronutrient concentration in soil at 45 DAS and at harvest. At 45 DAS boron content was highest in S_3. At harvest iron, zinc and manganese was highest in S_3 and was on par with S_4 while Mo was highest in S_3. Considering foliar spray of micronutrient formulation, iron, manganese, zinc, boron and copper in soil were in F_3 at 45 DAS. At harvest iron, manganese, copper, molybdenum and zinc were highest in F_3. In the interaction S_3F_3, manganese, zinc, copper and boron were higher at 45 days after sowing. At harvest iron and manganese recorded highest value in S_3F_3.
11. Seed treatment influenced the phosphorus and potassium content in leaf which was higher in seed treatment receiving micronutrient formulation at 2 %. Whereas, nitrogen content in leaf was not influenced by seed treatment.
12. Two foliar spray of micronutrient formulation increased the nitrogen, phosphorus and potassium content in cowpea leaves. In the interaction S_3F_3 recorded highest NPK content in cowpea leaves.
13. Treatments also influences the concentration of calcium and magnesium content in leaf. Foliar spray of micronutrient formulation increased the calcium content in leaf. Magnesium was found to be higher in treatment S_3F_3. However, sulphur content in leaf was found to be non significant.

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14. Seed treatment with 2 % micronutrient formulation recorded maximum concentrations of iron, manganese and zinc in cowpea leaves. Boron was highest in S_3. Double foliar spray of micronutrient formulation showed highest concentrations of Fe, Mn, Zn and B concentrations in cowpea leaf. In the interaction seed treatment receiving 2 % micronutrient formulation + two foliar sprays showed highest content of Fe, Mn and Zn in leaves.
15. Nitrogen content in grain was found to be highest in S_2 (seed treatment with rhizobium) while potassium was highest in S_3. Phosphorus concentration in grain was found to be non significant with respect to seed treatment. Foliar spray of micronutrient formulation at 15 DAS and 30 DAS showed highest concentrations of nitrogen and potassium in grain. In the treatment interaction nitrogen was higher in S_2F_3 (seed treatment with rhizobium + two foliar sprays) and potassium in S_3F_3. Similarly, the uptake of N, P and K was higher in seed treatment with 2 % micronutrient formulation and two foliar sprays. P and K uptake in the interaction was higher in S_3F_3.
16. Calcium content in grain was higher in S_3, F_3 and S_3F_3 and its uptake also followed the same trend. Magnesium and sulphur content in grain was found to be non significant with respect to different treatments while its uptake was higher in S_3 and F_3.
17. The concentration of micronutrients viz., Fe, Mn, Zn, Cu and B were found to be higher in seed treatment with micronutrient formulation at 2 %, double foliar spray of micronutrient formulation and their interactions. Uptake of Fe, Zn and B was higher in seed treatment with micronutrient formulation at 2 %, double foliar spray of micronutrient formulation and their interactions. While Mn uptake was higher only at interaction S_3F_3.

Conclusion

The results obtained from the investigation revealed that the application of micronutrient formulation as foliar spray (15 and 30 DAS) along with seed treatment (2 % micronutrient formulation) was found to be highly effective in increasing the growth and yield characters of cowpea. The uptake of nutrients in grain was also found to increase with the application of micronutrient formulation enhancing the nutritional quality of grain. Considering the benefit cost ratio treatment T_9 (seeds treated with micronutrient formulation at two percentage along with two foliar spray) was found to be the most effective and profitable method.

Future line of work

- Micronutrient seed treatment along with rhizobium inoculation can be tried in cowpea
- Effect of other varieties and hybrids are to be studied for knowing crop performance to micronutrient formulation

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EVALUATION OF MICRONUTRIENT FORMULATION IN COWPEA

(*Vigna unguiculata* L. Walp)

by

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(2017-11-144)

Abstract of the thesis

Submitted in partial fulfilment of the

Requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY

COLLEGE OF AGRICULTURE

PADANNAKKAD, KASARGOD - 671 314

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2019

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ABSTRACT

The project "Evaluation of micronutrient formulation in cowpea (*Vigna unguiculata* L. Walp)" was carried out at College of Agriculture, Padannakkad and Regional Agricultural Research Station (RARS), Pilicode during 2017 to 2019. The objectives of the study were to evaluate the effect of micronutrient formulation through seed treatment and foliar nutrition on growth, nodulation and yield of cowpea and to study its effect on plant nutrient uptake and residual soil nutrient status.

Laboratory study was carried out at COA, Padannakkad to formulate and standardize the micronutrient solution for cowpea. Different concentrations of the standardized micronutrient formulation were used for seed treatment study (Experiment 1). The experiment was carried out in completely randomized design with 7 treatments and 3 replications which included seed treatment with micronutrient formulation @ 0.25, 0.50, 0.75, 1, 1.5 and 2 per cent as T_1, T_2, T_3, T_4, T_5 and T_6 respectively. T_7 (seed treatment with water) was the control. Observations on germination percentage, number of days taken for germination, seedling length at three leaf stage and seedling vigour index were recorded. Treatment T_6 (micronutrient formulation @ 2 %) showed highest seedling length (24.23 cm) and seedling vigour index (2423) which was followed by T_5 (micronutrient formulation @ 1.5 %). Thus the best and second best treatment from seed treatment study was concluded as T_6 and T_5 respectively and was selected for the field experiment (Experiment 2).

The field experiment was conducted in randomized block design with 12 treatments replicated three times. The treatment consisting of combination of four levels of seed treatment (no seed treatment, seed treatment with rhizobium, seed treatment with best concentration from experiment 1 and second best seed treatment from experiment 1) and three levels of foliar application of micronutrient (no foliar application, one foliar application at 15 DAS and two foliar applications at 15 and 30 DAS). Biometric observations were recorded at flower bud initiation and at harvest.

Plant height at flower bud initiation and at harvest was superior in S_3 (Seed treatment with micronutrient formulation at 2 %) while stem diameter was highest in S_4 (seed treatment at 1.5 %) and S_3 respectively in both stages. Double foliar spray was superior in plant height and stem diameter at both stages. Interaction S_3F_3 (2 per cent seed treatment plus two foliar spray) recorded highest plant height at both stages and stem diameter was highest in S_2F_3 (treatment with rhizobium plus two foliar spray). Number of nodules was highest in S_2 (seed

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treatment with rhizobium). Number of pods per plant (21.02 & 20.97), pod weight per plant (36.14 g & 33.49 g), grain yield (2224 kg ha^{-1}) and dry matter production (2292.91 & 2372.92 kg ha^{-1}) was superior in S_3 and F_3 (two foliar spray). Interaction S_3F_3 was superior in number of pods per plant (25), pod weight per plant (38.11 g), grain yield (2320 kg ha^{-1}) and dry matter production (2613.24 kg ha^{-1}).

Leaf analysis revealed that P and K content in leaf was highest in S_3. N, P, K and Ca content in leaf was highest in F_3. Interaction S_3F_3 recorded highest N, P and K content in cowpea leaf. Mg content was highest in S_2F_3 (treatment with rhizobium plus two sprays). Fe, Mn, Zn and B content in leaf was highest in S_3 and F_3. Interaction S_3F_3 (2 per cent seed treatment plus two foliar spray) recorded highest Fe, Mn and Zn content in cowpea leaves. The uptake of N, P, K, Ca, S, Fe, Mn, Zn and B were highest in both S_3 and F_3 whereas Mg uptake was highest in S_3 and F_2 (one foliar spray). Cu uptake was highest in F_3. Interaction S_3F_3 recorded highest uptake of N, P, Mn and Zn. N content in cowpea grain was superior in S_2 and F_3. While K, Ca, Fe, Mn, Zn, Cu and B content in cowpea grain was highest in both S_3, F_3 and interaction S_3F_3. In case of interaction, N was highest in S_2F_3. The uptake of N, P, K, Ca, Mg, S, Fe, Zn and B in cowpea grain was highest in both treatments S_3 and F_3 and the interaction, S_3F_3 recorded highest uptake of P, K, Ca, Fe, Mn, Zn and B in grain.

Studies on residual soil nutrient status revealed that available N and P in soil was highest in S_2 at both 45 DAS and at harvest. Available K was highest in S_3, F_3 and S_3F_3 at both stages. Organic carbon content was found to increase in plots receiving two foliar spray and interaction S_3F_3. Available Fe, Mn and Zn was highest in S_3 at harvest and in F_3 at both stages. Available B was highest in S_3 and F_3 at 45 DAS while Mo at harvest. B and Zn were highest in S_3F_3 at 45 DAS while at harvest available Fe was found to be higher in S_3F_3. Available Fe at 45 DAS was highest in S_1F_3 (no seed treatment plus two foliar spray). Root CEC was superior in S_3, F_3 and in interaction S_3F_3.

The results obtained from the investigation revealed that the application of micronutrient formulation as two foliar spray (15 and 30 DAS) along with seed treatment (2 per cent) was found to be highly effective in increasing the growth and yield characters of cowpea. The uptake of nutrients in cowpea leaf and grain was also found to increase with the application of micronutrient formulation enhancing the nutritional quality of grain.

APPENDIX I

Daily average weather parameters of RARS, Pilicode

Date	Temperature		Relative humidity		Rainfall (mm)
	Max	Min	I	II	
19-10-2018	30	25	92	70	1.8
20-10-2018	30	24	96	81	21.8
21-10-2018	28.9	23.9	88	69	0
22-10-2018	30.5	24	92	70	0
23-10-2018	30.5	24.5	91	63	0
24-10-2018	31.5	23.5	91	57	0
25-10-2018	32	23.5	88	59	0
26-10-2018	32	24	80	49	0
27-10-2018	33	24	91	56	0
28-10-2018	32.5	22.5	83	47	0
29-10-2018	33	22	91	54	0
30-10-2018	32.5	21	85	48	0
31-10-2018	32	21.5	88	53	0
01-11-2018	32	24	91	64	0
02-11-2018	31.5	25	92	58	0
03-11-2018	32.5	25	93	71	0
04-11-2018	31	25	92	63	8.2
05-11-2018	32	25	95	69	0
06-11-2018	31.5	25	90	58	0
07-11-2018	32.5	25	88	63	0
08-11-2018	32	23	87	54	0
09-11-2018	31.5	23	84	52	0
10-11-2018	32.5	24	87	61	0
11-11-2018	32	23	91	66	0
12-11-2018	31.5	23	87	60	0
13-11-2018	31.5	23.5	86	60	0
14-11-2018	32	21.5	91	56	0
15-11-2018	31.9	19.5	91	47	0
16-11-2018	32.5	23.5	83	63	0
17-11-2018	31.5	23.5	91	58	0
18-11-2018	32	25	88	63	0
19-11-2018	31.5	25.5	96	73	0
20-11-2018	30.9	24.5	92	68	0
21-11-2018	30.5	25.5	92	65	0
22-11-2018	32.5	25.5	88	60	0
23-11-2018	32.5	25	93	63	0

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24-11-2018	32	24	94	69	6.8
25-11-2018	31.5	24	92	69	40
26-11-2018	31.5	24	96	66	0
27-11-2018	31.5	24	85	63	0
28-11-2018	32	21.5	87	46	0
29-11-2018	32	20.5	93	55	0
30-11-2018	32.5	25	88	66	0
01-12-2018	32	24	92	67	0
02-12-2018	31.5	24	91	58	0
03-12-2018	32.5	21.5	92	64	0
04-12-2018	31	22	87	63	0
05-12-2018	31	23.5	90	63	0
06-12-2018	31.5	20.5	93	70	0
07-12-2018	31.6	21.8	93	66	0
08-12-2018	32.2	23	91	63	0
09-12-2018	32	23	91	60	0
10-12-2018	32	23.5	91	73	4.2
11-12-2018	30	22	92	66	0
12-12-2018	31.4	23.5	91	66	0
13-12-2018	30.3	21	91	60	0
14-12-2018	31.5	21.2	91	66	0
15-12-2018	31.5	20.5	91	66	0
16-12-2018	32	21	95	66	0
17-12-2018	31	19	90	53	0
18-12-2018	30.5	17.7	93	70	0
19-12-2018	32.5	18.5	89	70	0
20-12-2018	31.5	23	93	61	0
21-12-2018	31	22	93	63	0
22-12-2018	31.2	21.8	88	59	0
23-12-2018	32	23.5	91	60	0
24-12-2018	32	24	96	66	0
25-12-2018	31	22.5	92	63	13.8
26-12-2018	32	23	91	66	0
27-12-2018	31.5	23	92	66	0
28-12-2018	31	22.5	91	66	0
29-12-2018	31	23	93	69	0
30-12-2018	30.5	23.2	92	66	0
31-12-2018	30.5	21	94	60	0

APPENDIX II

Interaction effect of seed treatment and foliar spray of micronutrient formulation on B : C Ratio

Treatments	Cost of cultivation	Gross return	B:C Ratio
T_1	83947	127600	1.52
T_2	100000	160000	1.60
T_3	107988	180340	1.67
T_4	86671	131740	1.52
T_5	100000	160000	1.60
T_6	104036	170620	1.64
T_7	105712	183940	1.74
T_8	110333	198600	1.80
T_9	110441	199900	1.81
T_{10}	104971	181600	1.73
T_{11}	106198	181600	1.71
T_{12}	113953	196000	1.72