PHYSIOLOGICAL EFFECT OF SEED PRIMING AND FOLIAR APPLICATION OF GROWTH REGULATORS IN COWPEA (*Vigna unguiculata* L.).

By

SWATHY P

(2019-11-207)

DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
PADANNAKKAD, KASARAGOD 671314
KERALA, INDIA

2022

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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 PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
PADANNAKKAD, KASARAGOD 671314
KERALA, INDIA

2022

DECLARATION

I, hereby declare that this thesis entitled **"Physiological effect of seed priming and foliar application of growth regulators in cowpea (*Vigna unguiculata* L.)"** 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 of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

24/5/2022

SWATHY P
(2019-11-207)

Place: Padannakkad
Date: 24/5/2022

CERTIFICATE

Certified that thesis entitled **"Physiological effect of seed priming and foliar application of growth regulators in cowpea (*Vigna unguiculata* L.)"** is a bonafide record of research work done independently by **Ms. SWATHY P (2017-11-207)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associate ship or fellowship to him.

Place: Padannakkad

Date: 24/05/2022

Sudarsana Rao 24/05/2022

Dr. G.V. Sudarsana Rao

Major Advisor

Professor and Head

Department of Plant Physiology

College of Agriculture

Padannakkad, Kasaragod

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. SWATHY P (2019-11-207) a candidate for the degree of **Master of Science in Agriculture** with major field in Plant Physiology, agree that the thesis entitled **"Physiological effect of seed priming and foliar application of growth regulators in cowpea (*Vigna unguiculata* L.)"** may be submitted by Ms. SWATHY P. (2019-11-207), in partial fulfilment of the requirement for the degree.

Dr. G.V. Sudarsana Rao 24/05/2022
(Chairman, Advisory committee)
Professor and Head
Department of Plant Physiology
College of Agriculture
Padannakkad.

Dr. Sumesh K.V. 24/05/22
(Member, Advisory committee)
Assistant Professor
Department of Plant Physiology
College of Agriculture
Padannakkad.

Dr. Binitha N.K. 24/5/22
(Member, Advisory committee)
Assistant Professor and Head
Department of Soil Science and Agricultural Chemistry
College of Agriculture,
Padannakkad

Dr. Jinsy V. S. 24/5/22
(Member, Advisory committee)
Assistant Professor
Department of Agronomy
College of Agriculture,
Padannakkad

ACKNOWLEDGEMENT

First and foremost, I bow my head before the ***Almighty God*** *who enabled me to successfully complete the thesis work.*

It is with great respect I avail this opportunity to express my deep sense of gratitude and indebtedness to my major advisor, ***Dr. G. V. Sudarsana Rao****, Professor, Department of Plant Physiology, College of Agriculture, Padannakkad for his meaningful guidance, Constructive suggestions, kind consideration, support, and wholehearted co-operation rendered throughout the course of my study. I really consider it my greatest fortune in having his guidance for my research work and my obligation to him lasts forever.*

I sincerely thank ***Dr. Sumesh K. V.,*** *Assistant Professor and Department of Plant Physiology, College of Agriculture, Padannakkad, for his timely suggestions and critical comments that helped to successfully complete my research work.*

I express my heartfelt thanks to ***Dr. Binitha N. K.,*** *Assistant Professor, Department of Soil Science and Agricultural Chemistry, College of Agriculture, Padannakkad for her timely advices, valuable guidance and critical comments that helped to successfully complete my research work.*

I extend my cordial thanks to, ***Dr. Jinsy V. S.,*** *Assistant Professor, Department of Agronomy, College of Agriculture, Padannakkad and member of my advisory committee for her constant support, practical suggestions and thorough evaluation of this manuscript.*

I have infinite pleasure to express whole hearted thanks for the innumerable help and support especially ***Dr. Rajesh Sir,*** *Assistant professor, Department of Plant Physiology, RARS Pillicode.*

I express my heartfelt thanks to Librarian, College of Agriculture, Padannakkad, ***Dr. Ajthakumari*** *and all other staff members of library, who guided me in several ways, which immensely helped for collection of literature for writing my thesis.*

I extend my cordial thanks to ***Dr. Sreekumar Sir, Dr. Sujatha R., Dr. Raji Vasudevan Namboodiri, Dr. Vyjayanthi, Dr. Manikandan, Lenina Mam****, who guided me in several ways, which immensely helped for collection of literature for writing my thesis.*

*I express my sincere thanks to my dear friend **Akshay Kumar** for his constant help and encouragement that made me confident in difficult situations.*

*I express my sincere thanks to my batchmates especially **Arya, Anjitha, Veena, Sreelakshmi, Sai Parvathi** for their help offered during my thesis work. My heartfelt gratitude cannot be explained in words for the constant support and affection of my dearest seniors and juniors especially **Fathimath Suhaila**, **Vishnu K. S**., **Lintu, Karishma** throughout my research work and course.*

*I sincerely thank for the guidance and facilities provided by **Suresh Sir**, Farm manager and **Ratheesh sir**, Farm officer, College of Agriculture, Padannakkad.*

I am in dearth of words to express my love towards my beloved family members for their boundless affection, moral support, eternal love, deep concern, prayers and personal sacrifices which sustain peace in my life.

I express my deep sense of gratitude to Kerala Agricultural University for financial and technical support for persuasion of my study and research work. It would be impossible to list out all those who have helped me in one way or another in the successful completion of this work. I once again express my heartful thanks to all those who helped me in completing this venture in time.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

ANOVA	Analysis of Variance
BR	Brassinolide
^{0}C	Degree Celsius
CGR	Crop growth rate
cm	Centi meter
CRD	Completely randomized design
DAS	Days after sowing
EC	Electrical conductivity
et al.,	Co-workers/Co-authors
Fig.	Figure
FYM	Farm yard manure
ds	Deci siemen
g	Gram
ha	Hectare
i.e.,	That is
KAU	Kerala Agricultural University
kg	Kilogram
kg/ha	Kilogram per hector
LAI	Leaf area index
m	Meter
mg	Milligrams
mM	Milli molar
pH	Power of hydrogen ion
POP	Package of practices
ppm	Parts per million
RBD	Randomized block design
RGR	Relative growth rate
RWC	Relative water content
SA	Salicylic acid
SLW	Specific leaf weight
SPAD	The soil plant analysis development
TU	Thiourea
viz,	Namely
%	Per cent
@	At the rate

Introduction

1. INTRODUCTION

Pulses are protein rich edible seeds consumed all over the world, having high nutritional value. Pulses are categorised into different types, based on its production and consumption. The major group of pulses include cowpea, chickpea, mung bean, pigeon pea, lentil, common bean, urd bean and dry bean, while rice bean, moth bean etc. are some minor pulses grown in different parts of world, like dry bean in India. In India, pulse production is lagging behind cereals (Singh, 2016). With low input, these crops are cultivated on marginal lands which decreases production and productivity (Viswanatha *et al.,* 2016). Improper management, seed rate, sowing methods, lack of proper seed treatment, variation in sowing time, gradual and sudden outbreak of pest and disease incidence, climate variation, insufficient irrigation are causing reduction of yield and quality of grains.

Cowpea (*Vigna unguiculata* L.) is a well-known crop cultivated all over the world for vegetable, grain and fodder purpose. It originated from West Africa and belongs to the family Fabaceae and subfamily Papilionaceous. Cowpea requireswarm, semi-arid weather for better establishment. Cold climate adversely affects yieldand quality of output. Well drained loamy soil is most preferred but difficult to establish in saline or alkaline soil. Cowpea is referred as black-eyed pea, crowder pea,southern pea etc. which is an annual crop having high nutritional value in grains. Immature pods are used for vegetable purpose. Cowpea seeds are rich source of proteins, carbohydrates, fats, dietary fibres, minerals like iron, calcium, phosphorus, potassium, magnesium, zinc, copper and various essential amino acids like lysine, leucine and phenylalanine (FAO, 2016).

To meet the nutritional requirement of diet and strengthening soil nutrient status production potential of pulse crops should be promoted. To achieve this, boosting present technology and following proper management should consider (Singh, 2016). To increase the production potential of cowpea, seed priming and foliar spray of growth regulators can be adopted. Seed priming is a pre-soaking treatment of seeds in nutrient or hormone solutions. It helps for faster germination and emergence of vigorous seedlings. Seed priming enhance metabolic activities inside seed which accelerate better establishment. Among various methods of fertilizer application, foliar spray of nutrients is one of the most effective methods, which helps for faster uptake of nutrients by plants compared to traditional method of soil application. Foliar spray of growth regulators increases the level of various biomolecules in plant leaves compared to control plants (Aslam *et al*., 2016).

Conventionally, there are five main classes of plant growth hormones which are used to alter plant growth and development. Auxin, cytokinin and gibberellin comes under growth promoters while ethylene and abscisic acid comes under growth inhibitors. Apart from these, plants also show response towards some biochemically synthesised biomolecules like brassinosteroids, salicylic acid, jasmonates, phenolics, alkaloids, polyamines etc. These chemicals play some specific role in plant growth regulations (Srivastava, 2002; Buchanan *et al*., 2015; Taiz *et al.*, 2015). To manipulate crop yield and quality these novel plant growth regulators are preferred at lower concentrations as an input for sustainable farming.

Salicylic acid is a naturally occurring compound coming under class of phenols, having significant role in plant growth and development. It plays important role in regulating various physiological functions in plants from germination to harvest, such as seed germination, leaf development, nutrient uptake, ion uptake, flower induction and longevity, thermogenesis, transpiration, stomatal movement, photosynthesis, synthesis of various biomolecules like chlorophyll, proteins, ethylene etc. Treatment of plants with salicylic acid at lower concentration provides tolerance against abiotic and biotic stresses (Idrees *et al.,* 2011; Jini and Joseph, 2017; Miuraand Tada, 2014) by regulating defensive response against various pathogens and maintain plant health (Vincente and Plasencia, 2011).

Brassinosteroids are a group of emerging steroidal hormone having wide range of application in agricultural crop production. Presence of a new group of lipoidal hormone, brassin in rape pollen was proposed by Mitchell *et al.,* (1970). Brassinolide and its associated compounds are together referred to as brassinosteroids. These are actually to polyhydroxy steroids, which helps for increased cell division, cell elongation and cell enlargement like other classes of growth regulators. One of the major function associates with it is tolerance to various abiotic stresses in different crops. Analogues of brassinosteroids including brassinolide, 24-epibrassinolide and 28-homobrassinolide are used even under field conditions due to the property of stability under such condition.

Thiourea, otherwise known thiocarbamide is a nitrogen and sulphur containing compound having wide range of role in agricultural crop improvement from seedling stage. Seed priming along with foliar spray of thiourea significantly enhances yield and quality of different crops under normal as well as adverse stress conditions. Foliarspray of thiourea break seed and bud dormancy and regulate flowering period in ornamentals (Chang and Sung, 2000).

Hence, the present study entitled "Physiological effect of seed priming and foliar application of growth regulators in cowpea (*Vigna unguiculata* L.)" is proposed with the objective:

- Elucidation of influence of seed priming and foliar application of growth regulators on morpho-physiological changes, yield and quality in cowpea (*Vigna unguiculata* L.).

Review of literature

2. REVIEW OF LITERATURE

Plant growth altered by various factors. Both internal and external factors can impart significant change in plant metabolism and this can be used as a tool to manipulate crop growth and yield. Among the factors, plant growth regulators are one of the important means to alter the morpho-physio and biochemical parameters of plant system. Plant growth regulators are chemical substances required by plants in small concentration and impart considerable effect on growth and development. They implicate rapid impact on plant response by altering metabolic processes, thereby enhancing better growth and yield.

2.1 IMPORTANCE OF COWPEA AS A MULTIPURPOSE CROP

Cowpea (*Vigna unguiculata* L.) is an important annual herbaceous crop cultivated all over the world for various purposes including as vegetable, grain, fodder, soil enrichment etc. Cowpea is a leguminous crop originated in Africa having wide variation in growth pattern, morphological behaviour, maturity etc. It exhibits epigeal germination with a pair of oppositely arranged young leaves at seedling stage, later transformed to profuse oblong shaped trifoliate leaves from main stem and branches. At reproductive stage, flowers of different colours with respect to species are emerged as racemose inflorescence from peduncle. Self-pollinated cowpea flowers produce seeds which are arranged serially in pods. The colour and length of pods varies with cultivar. Immature pods can be consumed as vegetable at green stage and as grain when it changes to yellowish ripe stage.

In diet, it is a good source of low-cost protein rich grain for human and as fodder for animal consumption. In some part of the world, it is consumed as leafy vegetable and some of them use green pods as such for table purpose. Cowpea is enriched with lysine, tryptophan and various antinutritional factors like trypsin inhibitors, phytate, oxalates, hemagglutinin, oligosaccharides and polyphenols (Afiukwa *et al.,* 2012; Sreerama *et al.,* 2012) while low in cysteine and methionine compared to cereals. Due to the presence of folic acid in grain, it is a good nutrient source to pregnant women. Hay made with cowpea leaves and stem are very good nutritive feed to livestock (Ravhuhali *et al*., 2011).

Cowpea enhances soil nutrient status through biological nitrogen fixation by symbiotic relations with soil rhizobia (Sarr *et al.*, 2015) along with this it uplift soil fertility by incorporating organic matter into the soil and hence improve the productivity of other crops in combination as a rotation crop. Deep taproot system of plants function as an adaptive mechanism to tolerate drought stress, in addition to this presence of expanded leaves help to reduce evaporative loss from soil and function as a cover crop in agricultural practices.

2.2 SEED PRIMING AND FOLIAR APPLICATION OF GROWTH REGULATORS

Seed germination and seedling emergence are important events in plant life cycle. Poor emergence of seedling along with insufficient establishment are the main problems faced by farmers (Singh *et al.,* 2015). To overcome these problems several reports highlighting the positive effect of pre-treatment of seeds using various growth regulators. Among the methods seed priming is a low-cost technique adopted in farming practice in order to enhance metabolic processes inside the seeds before it germinates.

2.2.1 Effect of seed priming on growth and development of plant

Seed priming is a physiological seed strengthening process. It is a method of hydro or osmotic priming process that enhances germination of seeds and reduces the time taken for germination (Zhu *et al.,* 2021). It shortens time for emergence of rice seedlings, where shoot length and dry weight were higher in seeds subjected to priming compared to water soaked and controlled seed (Matsushima and Sakagami, 2013). It is a better way to reduce time for seedling emergence, for uniform seedling development and enhancing seedling vigour (Ashraf and Foolad, 2005). Here seeds are hydrated, then subjected to redrying to its normal condition before it used for sowing (Farooq *et al.,* 2019). During seed priming alpha amylase activity is enhanced which alter various metabolic activity in seeds which ultimately increased the vigour of seedlings (Lee and Kim, 2000). Priming of seeds enhances early and uniform germination of seedlings (Jisha *et al.,* 2013), and improve yield (Harris *et al.,* 2007). This pre-treatment enhances germination of seedlings along with increased the level of various compounds which counteract stress in plants (Wang *et al*., 2017). Osmo-priming of clover seeds for eight and 16 hours increased the vigour index over control plant (Rouhi *et al.,* 2010)

Seed priming with brassinolide enhances germination and initial growth of seedlings. The treatment helps to liberate plant hormones like auxin and GA in ground nut seedlings under water stress condition and increased the level of ABA and SA and enhance mechanism under drought (Vardhini and Rao, 1998). Low (0.004 mg L^{-1}) and high (0.4 mg L^{-1}) concentration brassinosteroid enhances germination capacity of pine seeds while at medium (0.04 mg L^{-1}) concentration, germination percentage get retarded (Cukor *et al.,* 2018).

Study of effect of homobrassinolide by seed treatment and foliar spray on wheat under irrigated and water stressed condition showed that the treated plant exhibited better growth, photosynthetic functioning, nitrate reductase activity and final yield compared to non-treated plants. Treated plants had better membrane stability under moisture stress condition (Sairam, 1994).

Imidacloprid toxicity on *Brassica juncea* L. was ameliorated with pre-soaking of seeds at different concentration of 24-epibrassinolide, where plants grown under toxicity showed reduction in vegetative growth, photosynthetic pigments, gaseous exchange rate etc. while the plants which were grown from pre-soaked seeds showed better vegetative growth, enhanced biosynthesis of photosynthetic pigments, and decreased toxicity effect of pesticide by efficient functioning of antioxidant system. The best result was observed when 100 nM BR solution was used for priming of seeds (Sharma *et al*., 2016). Similarly, Cr toxicity in rice alleviated by priming seeds with 0.01 μM brassinosteroids. Germination quality along with vigour index were enhanced through the pre- treatment of seeds (Basit *et al.,* 2021)

Semida and Rady (2014) primed common bean seeds with 5.0 µM 24-EBR or in 1.0 mM SA and allowed to grow in salt stressed condition. Even under adverse salinity, seedlings showed better growth, seed germination percentage, osmotic adjustment, membrane stability, reduced oxidative stress by adjusting anti oxidative system and helped the plant to overcome adverse effect of salt stress.

According to Farooq *et al.,* (2008) chilling injury of maize can overcome by seed priming with salicylic acid, the best response was observed when seeds primed with 50 mg L^{-1} SA, it enhanced the antioxidant mechanism in plants and imparted chilling stress tolerance in it.

Singh and Singh (2016) have shown that under temperature stress, tomato seeds when primed with 0.5 mM salicylic acid imparted higher germination percentage and seedling growth compared to unstressed plants. Here minimum number of days taken for seed germination also enhanced the reproductive characters. According to El-Wakil *et al.* (2017) pre-treatment of potato seedling with salicylicacid enhanced seedling survival and made less susceptible to soil borne pathogens.

Hemandez-Nistal *et al.,* (1983) reported that treatment of *Cicer arietinum* L. with thiourea increases K^+ mobilisation and enhances dark rection which ultimately accelerate growth of embryonic axis. Thiourea enhances ion transport in embryonic stage which accelerate germination and seed dormancy over came with treatment (Aldasoro *et al.,* 1981).

2.2.2 Effect of foliar application on growth, development and yield of plants

Foliar spray is one of the ways of chemical application, where plants are nourished directly on photosynthetic surface, hence it helps the plants not only for quick uptake of nutrients but also having longer life compared to solid form of nourishment (Katiyar *et al.*, 2015). Younger leaves are more responsible for faster uptake of nutrients than matured leaves (Sargent and Blackman, 1962). Foliar application of growth regulator significantly enhanced reproductive character and productivity in okra, here chemical compounds are required only in lesser quantity and it exhibited high efficiency of nutrient uptake compared to direct soil application.

Henceforth, it acts as a quick means for enhancing crop growth and yield (Dev *et al.,* 2017).

Pegu *et al.* (2020) reported that foliar application of nitrogen had significant effect on various morpho-physiological parameters of rapeseed compared to feeding of solid fertilizers. The highest leaf area, specific leaf weight and total chlorophyll content were recorded with foliar spray of urea (1% and 2%) at 20 and 40 DAS along with recommended dose of NPK compared to NPK soil application only. Yield parameters like number of siliquae per plants were higher when two foliar fertilization of urea (1% and 2%) were given along with recommended dose of NPK (102.13 and 104. 8, respectively.) over control NPK alone (90.13).

2.3 SIGNIFICANCE OF PLANT GROWTH REGULATORS

2.3.1 BRASSINOSTEROIDS

Brassinosteroids are new class of steroidal plant hormone having important function in plant growth and development. It plays important role in increasing the rate of protein synthesis, hence increased the nucleic acid level in plant cells. So, protein content was higher in plants subjected to brassinolide treatment (Bajguz, 2000). Mitchell *et al.*, (1970) identified the lipoidal hormone brassin from pollens of Brassicaceae family. Brassinosteroids contain group of brassinolides and its related compounds. Many reports are highlighting the positive impacts of the compound on growth, development and yield attributes on different plants. Enhanced cell division and cell elongation accelerated the growth of plants just like functions of other class of growth regulators. Imparting tolerance to various abiotic stresses in crops under adverse stress conditions is another important function of brassinosteroids. 24- epibrassinolide (24-EBR), 28-homobrassinolide (28-HBR) and brassinolide are commonly used brassinosteroids. Due to its property of stability under varying conditions, it can also be recommended under field conditions.

2.3.1.1 Consequence of brassinosteroids on morphological parameters

Many workers reported the effects of brassinosteroids on morphological parameters of different crops. Asha and Lingakumar (2015) reported that foliar application of 24-EBR at 1.0 μM concentration on cowpea at seedling stage exhibited better performance of vegetative characters like root length, shoot length, leaf area, fresh and dry weight of shoots. According to Vardhini and Rao, (1998) BR and 24-EBR at 3 μM concentration gave best results on groundnut shoot length (25.6 cm and 26.4 cm respectively), fresh weight (54.8 g and 61.34 g respectively) and dry weight (12.83 g and 13.24 g respectively) of seedlings compared to the same on untreated plants (16.4 cm, 20.23 g and 7.91 g respectively). Root length, fresh and dry weight were increased on treatment with 24-EBR @ 3 μM (7.2 cm, 9.7 g and 6.1 g respectively) and BR @ 3 μM (13.14 cm, 2.4 g, and 0.99 g respectively) over control (7.1 cm, 1.78 g and 0.68 g respectively).

According to Franck *et al.* (1998) *in vitro* culture of sweet pepper for direct organogenesis, media supplied with 24-EBR at 0.1 μM concentration exhibited enhanced stem elongation. Matwa *et al.* (2017) confirmed that BR at 0.25 ppm foliar spray on green gram increased number of flowers (39.3) compared to control (27.7), also flower to fruit ratio was significantly superior (72.67) than plants which were not treated (52.33).

2.3.1.2 Consequence of brassinosteroids on physiological parameters

Many workers have testified the effect of brassinosteroids on physiological parameters of different crops. According to Sengupta *et al.* (2011) twice the application of BR at 0.25 ppm concentration on green gram under moisture stress condition showed increased pod yield due to cumulative increase of plant height, leaf area index and dry matter accumulation, where the highest LAI (4.94) reported at 45 DAS with 0.25 ppm BR spray. They supposed it due to BR induced the action in biosynthesis of IAA and GA in plants. Coban and Baydar, (2016) observed that increase in both fresh and dry weights of aerial parts and whole dry leaf weight of pepper mint treated with brassinosteroids at varying concentration, the best result was obtained when brassinosteroid sprayed at 0.25 mg L^{-1}.

Induced boron toxicity in *Vigna radiata* L. was overcame by subsequent application of 28-HBR. Under such stress conditions these steroidal hormones enhanced the activity of antioxidant enzymes which imparted tolerance in plants against boron toxicity, also improvement of growth, relative water content and enhanced photosynthetic rate were achieved (Yusuf *et al.,* 2011). Plants treated with BR at 10^{-10}, 10^{-8}, or 10^{-6} on *Vigna radiata* showed increased SPAD chlorophyll meter value (17.8%, 29.9% and 10.9% respectively) over control (Alyemeni and Al- Quwaiz, 2016). According to Yu *et al.,* (2004) EBR treated cucumber plants exhibitedhigher PS II quantum yield over control, the value attained at 0.45 with in one day. Also, Fv/Fm value reported the same improvement on EBR treatment. Pre-treatmentof chilling stressed cucumber seedlings with 28-HBR enhanced the activity Rubisco enzyme in chloroplast which maintained photosynthetic rate and maximum quantum yield of photosystem II (Fariduddin *et al.,* 2011).

2.3.1.3 Consequence of brassinosteroids on biochemical parameters

Many explorations have reported on effect of brassinosteroids on biochemical parameters of different crops. External application of 24-EBR on groundnut increased the level of nucleic acid in plant tissue enhanced rate of protein synthesis. Level of DNA and RNA molecules were higher when it was treated at 1 μM compared to 0.5 μM and 3 μM concentrations (Vardhini and Rao, 1998).

In peppermint, salt stress significantly raised the level of proline content, while reduction in proline content observed when it was treated with brassinosteroid at 1.5 mgL^{-1} and enhanced the antioxidant system in treated plants compare to the control (Coban and Baydar, 2016).

Three day continued foliar spray of 24-EBR, 1 μM at early stages of *Vigna unguiculata* L. showed that characteristic improvement in level of photosynthetic pigments on fresh weight basis, accumulation of chlorophyll *b* was more compared to chlorophyll *a* in freshly harvested leaves, similarly carotenoid content also increased after treatment. Lower level of proline in treated plant compared to untreated plants indicated reduced stress in plants subjected to BR application. Higher level of soluble protein was recorded when plants were treated with 1μM 24-EBR (Asha and Lingakumar, 2015).

Niu *et al.* (2016) conducted a study on exogenously applied BR at different concentration (0.01, 0.1 and 1.0 mg L^{-1}) on *Leymus chinensis* in relation with high and room temperature condition. The results showed that at room temperature the level of proline was less while foliar applied BR (1.00 mg L^{-1}) increased the proline content in plants (52.54 μg g^{-1}) compared with untreated plants (35.72 μg g^{-1}). In case of temperature stressed plants, BR spray @ 1.0 mg L^{-1} significantly enhanced the level of proline (622.07 mg L^{-1}) and reduced the negative impact of stress over control (111.74 mg L^{-1}).

2.3.1.4 Consequence of brassinosteroids on yield and quality attribute

Several workers have investigated the effect of brassinosteroids on yield and quality attributes on different crops. Matwa *et al.* (2017) reported that, when 0.25 ppm BR was applied on green gram before flowering, it showed a greater number of pods (28.7) compared to control (14.3). Pod length and pod weight were higher in plants treated with 0.25 ppm BR which resulted an increase in harvest index of crop (39.43) than control (22.43). Application of BR derivatives enhanced yield and quality of tomato under field conditions (Vardhini and Rao, 2001). Similarly, the rate of protein synthesis and nucleic acid content were enhanced by external application of 24-EBR which enhanced the yield and fat content in ground nut seeds (Vardhini and Rao, 1998).

Ritti *et al.* (2019) demonstrated that foliar spray of boron @ 1 g L^{-1} along with HBR @ 0.5 ml L^{-1} three times on bottle gourd female plants imparted significant increase in yield attributes. Fruit length (42.7 cm), fruit weight (760.7 g), fruit diameter (28.4 cm) and number of seeds per fruit (443.1) were higher in treated plantsover control plants (36.6, 672.8 g, 27.1 cm and 435.6, respectively), which ultimately increased crop yield, here it implied the response of HBR along with boron in accumulation of stored reserve.

2.3.2 SALICYLIC ACID

Salicylic acid (SA) is an emerging well known novel plant growth regulator which comes under the category of phenolic compounds. Salicylic acid is chemically an ortho-hydroxy benzoic acid having aromatic ring containing hydroxy functional group. It exists as a crystalline powder in its free state moderately soluble in water (Raskin, 1992). Basically, these are secondary metabolites derived from different organisms including plants and imparting defensive response against biotic and abiotic stresses (Idrees *et al.,* 2011; Jini and Joseph, 2017; Miura and Tada, 2014).

Regarding biosynthesis of SA, it involved in two major pathways from a common precursor, namely chorismic acid viz. phenylalanine ammonia-lyase (PAL) pathway and isochorismate (IC) pathway, among this PAL is primarily accountable for the synthesis of SA. It proposed that SA formed in chloroplast directed later to the cytoplasm (Hasanuzzaman *et al.,* 2017).

SA impart various physiological, biochemical and productive response in different plants at varying concentrations (Arberg, 1981). It plays major role in plant growth and development from seed germination (Cutt and Klessing, 1992) to yield characters by altering various plant growth constraints (Khan *et al.,* 2003) like enhanced photosynthesis (Fariduddin *et al.,* 2003), nutrient uptake and distribution, flower induction (Hayat and Ahamad, 2007) and enhanced flower longevity by altering ethylene biosynthesis (Leslie and Romani, 1986) etc.

2.3.2.1 Consequence of salicylic acid on morphological parameters

Many workers have demonstrated the morphological changes imparted by SA on different crop. Foliar spray of SA at different concentration on cowpea plants showed enhanced vegetative growth. However, the best result was obtained at 100 ppm concentration, plant height (58.3 cm), number of compound leaves per plant

(12.6), fresh weight of shoot per plant (62.08 g) and dry weight of shoot per plant (14.49 g) showed better response compared to control (44.3 cm, 7.3, 37.72 g, 7.63 g respectively). The anatomical observation revealed increased diameter of stem and leaf blade which implied the active growth of vascular structures (Azoz and El Taher, 2018).

Khandaker *et al.* (2011) reported that higher plant height, root length, dimensions of widest leaf, number of leaves, fresh and dry weight on red amaranth with the treatment of SA. Number of branches and leaves were higher in tomato plants subjected to the treatment in combination with vitamin E (Mady, 2009). According to Kazemi (2014) maximum plant height (120.31 cm) was obtained with SA treatment at 0.5 mmol L^{-1} on tomato.

There was significant increase in various morphological characters of lupine plants when it was treated with SA at 25, 50, 75, 100, and 150 ppm concentrations under two seasons. The suggested best dose of 75 ppm had more plant height (33.8% and 22.3%), number of primary branches per plant (34.88% and 36.6%), number of leaves per plant (21% and 33.3%) and total leaf area per plant (54.9% and 52.2%) in two seasons respectively over untreated plants (Gomaa *et al.,* 2015). According to Sadeghipour and Aghaei, (2012) seed priming of common bean at 0.5 mM SA enhanced the plant height during the growth period under normal conditions as well as under drought.

2.3.2.2 Consequence of salicylic acid on physiological parameters

Many studies reported the effect of SA on physiological parameters on different crops. Sadeghipour and Aghaei, (2012) reported that seed priming with SA at 0.5mM increased the LAI by 29 per cent in common bean under drought. According to Tarighaleslami *et al.,* (2012) 400 mM foliar spray of SA on maize increased the LAI. Combined application of SA and vitamin E imparted greater leaf area and dry matter production of tomato plant (Mady, 2009). Kazemi (2014) reportedan increased dry weight by 65.55% and maximum chlorophyll content (25 SPAD) were obtained with application of 0.5 mM SA compared to non-treated tomato plants.

Foliar application of SA @ 100 ppm on maize plants under water stressed conditions showed significantly higher leaf relative water content (79.37%) over untreated stressed plants (52.27%) (Rao *et al.,* 2012). In another study conducted by Edupuganti *et al.* (2019) on chickpea plants demonstrated that without any treatments, drought stressed plants showed decrease in RWC in leaves by 37.42 per cent over non-stressed plants. Under drought, foliar application of SA at 0.5 mM concentration improved it by 49.4 per cent in stressed plants and 55 per cent in irritated plants over check.

According to Hussein (2015) priming of okra seeds with varying concentrations of SA (10, 25, 50, 75 and 100 mg L^{-1}) before sowing improved the relative growth rate. Foliar application of SA at lower concentration (10^{-5} M) increased the dry matter accumulation in mustard, while with increasing the concentration of the spray from the specified concentration, imparted negative effect on plants (Fariduddin *et al.,* 2003).

Salicylic acid treated mango plants noted less incidence of mango fruit fly due to females laid less egg, reduced larval development and adult emergence. The treatment reduces females' preference towards oviposition in fruits due to some repellent compound in treated fruits (Damodaram *et al*., 2015).

2.3.2.3 Consequence of salicylic acid on biochemical parameters

Several studies reported the effect of SA on biochemical parameters. According to Khan *et al.* (2003) foliar application of SA on corn at 10⁻7 mol L $^{-1}$ stimulated photosynthetic rate by 8-13 per cent, lowering of intercellular CO_2 by sequestration in chloroplast indicated efficient photosynthetic activity compared to control. Azoz and El Taher (2018) investigated the effect of foliar spray of SA at 25, 50, 75, 100 ppm concentrations on cowpea plants showed increased photosynthetic pigments including chlorophyll *a*, *b*, total chlorophyll and carotene. The best results reported at 100 ppm (1.49, 0.47, 1.96, 0.28 respectively) compared to non-treated plants (1.27, 0.29, 1.56, 0.15 respectively). Similarly, the level of primary elements like N, P, and K in treated leaves were higher at 100 ppm (31.16, 40.0 and 50.81%) than control.

Khandaker *et al.* (2011) found that SA was effective to increase various bioactive compounds in red amaranthus. Chlorophyll and betacyanin content, total phenolic level and antioxidant activity were higher in treated plants compared to control. Mady (2009) revealed the effect of SA along with vitamin E in tomato improved the level of photosynthetic pigments like chlorophyll *a*, *b* and carotene. Regarding various mineral content in treated plants, combined application showed increased level of N compared to control and separate treatment, as well, crude protein was higher in plants subjected with the treatment of SA (50 ppm) + vitamin E (200 ppm), which indicated the increased leaf area and photosynthetic activity. Endogenous level of auxin became reduced, whereas the level of gibberellin and cytokinin were increased in all the treatments compared to non-treated plants. Maximum chlorophyll content and leaf nitrogen (2.54 %) were higher with application of 0.5 mM SA compared to non-treated tomato plants (Kazemi, 2014).

Gomaa *et al.* (2015) conducted an experiment on foliar application of SA at different concentrations on lupine. The results revealed that 75 ppm SA foliar application promoted the level of chlorophyll *a* (3.77 mg g^{-1}), chlorophyll *b* (1.531 mg g^{-1}) and carotenoids (1.615 mg g^{-1}) over control (3.129 mg g^{-1}, 1.313 mg g^{-1} and 1.411 mg g^{-1} respectively).

Souri and Tohidloo, (2015) determined that application of SA @ 100 mg L^{-1} as foliar and pre-root treatment on salt stressed tomato plants increased the level of proline content of leaves from the initial concentration, whereas in controlled non stressed plants there were no rise in proline content. Free proline present in lentil under salinity was higher when it was subjected to 100 mM NaCl + 0.5 mM SA, compared to root, shoot exhibited higher proline oxidase activity under saline condition (Misra and Saxena, 2009).

2.3.2.4 Consequence of salicylic acid on yield and quality attribute

Many investigations have demonstrated the effect of salicylic acid on yield and quality attributes of different crops. Azoz and El Taher (2018) reported that foliar application of SA significantly enhanced the number of pods per plant, seeds per pod, seeds per plant, weight of 100 seeds (g) and seed yield (g) cowpea, 100 ppm gave the best result 7, 32, 10 and 12 % respectively over non-treated plants.

Foliar spray of SA at different concentrations increased yield of red Amaranthus by increasing in leaf number and size, the best results recorded at 10^{-7} Mconcentration along with increased yield attributes, various biomolecules imparting nutritive value also increased with the treatments (Khandaker *et al.,* 2011). SA at 10^{-2} M concentration had best response in terms of quantity and quality. Vitamin C and lycopene content were higher in treated fruits compare to control. (Javaheri *et al.*, 2012).

Kazemi (2014) reported that the highest fruit yield (170.32 mg ha^{-1}) and mean fruit weight (95.94 g) were obtained from tomato with foliar spray of 0.25 mM Methyl jasmonate + 0.5 mM SA, followed by 0.5 mM SA with fruit yield (165.12 Mg. ha^{-1}) and mean fruit weight (95.6 g), similarly, substantial increase in level of TSS (6°Brix), TA (3.59 %) and Vit. C (15.14 mg/100 g fresh $fruit^{-1}$) recorded with the same combinations.

Foliar spray of SA at varying concentrations and at different frequency of application on cluster bean showed difference in vegetative character as well as yield attributes. Increased frequency of application increased yield of crops, while higher concentration did not have considerable effect on yield attributes (Shehata and Fahmy, 2019). Foliar spray of SA 10 ppm on mung bean increased pod yield and number of grains per pod under field conditions (Singh and Kaur, 1980).

Gomaa *et al.* (2015) reported that an increase in yield and yield parameters of lupine plants when applied with SA at different concentrations. At 75 ppm foliar spray number of pods per plants, seed per pod were more and increased seed weight over control and crude protein content in treated plant seed (22.5 %) was higher than control.

2.3.3 THIOUREA

Thiourea (TU) also called thiocarbamide is a growth regulator used for improving plant growth and development under stressed condition as well, it is a nitrogen-sulphur compound well soluble in water. Priming, foliar nourishment, soil application, media feeding are the practices adopted to improve productivity even under drought conditions. It enhanced plant growth and encouraging defensive metabolism in plant system (Sahu *et al.,* 1993). Thiourea hastens germination of seeds(Liacos and Nord, 1961) with great influence on axial growth (Esashi and Katoh, 1977).

2.3.3.1 Consequence of thiourea on morphological parameters

Many researches have done works to demonstrate the morphological effect of thiourea on different crops, few of them are reviewed here. According to Sahu *et al.* (1995) seed soaking (500 ppm) and foliar spray (1000 ppm) of TU on maize enhanced number of green leaves formation (9.5) over untreated plants (6.4), significant increase in plant height (12.75 %) also reported.

Premaradhya *et al.* (2018) reported that foliar spray of TU at 1000 ppm on lentil effectively increased shoot length (34.1 cm) over control, branching got flourished which ultimately improved crop canopy. Correspondingly, exogenous application of TU @ 1000 mg L^{-1} influenced various growth parameters of faba bean. Increased plant height, number of leaves per plant, branches and pods were highlighted the treatment, the increment recorded maximum at combined application of TU (1000 mg L^{-1}) given with aspartic acid 150 mg L^{-1} followed by single TU spray(Amin *et al.,* 2014).

According to Uddin *et al.* (2020) application of TU (1000 ppm) thrice on okra showed significantly increased plant height (131.5 cm) and had greater number of leaves (41.7) in treated plants over zero, once and twice application of chemicals. The same trend was also observed in maximum number of branch emergence on three times spray of TU. Ravat and Makani (2015) claimed that foliar spray of TU @ 500 and 1000 ppm on okra exhibited significant increase in number of leaves at its 90th day of growth stage by 41.1% and 26.34% respectively over untreated plants, the number of internodes also higher in treated plants.

2.3.3.2 Consequence of thiourea on physiological parameters

Many workers have reported the physiological effect of thiourea on different crops. Sahu *et al.* (1995) declared that foliar application of 1000 ppm TU gave 32.48 per cent more LAI in maize over control. Combined effect of seed soaking and foliar application were more effective on physiological characters of treated plants. Likewise, in okra application of TU @ 1000 ppm thrice enhanced the SPAD chlorophyll value, which ultimately increased the photosynthetic rate (Uddin *et al.,* 2020). Similar observations were recorded by Kaya *et al. (*2015) where seed treatmentalong with exogenous foliar application of 500 ppm TU enhanced the level of *Fv/Fm* value and total chlorophyll content of maize plants under salinity.

According to Amin *et al.* (2014) exogenous application of TU (1000 mg L^{-1}) increased the value of fourth leaf area, LAI, SLW and CGR of faba bean. Foliar nutrition with TU at 500 ppm on okra had positive effect in leaf area (46.66%) and LAI (34.14%) compared to control plants, also the treatment imparted earliness to flowering and increased the total dry matter production in both the trials (Ravat and Makani, 2015).

2.3.3.3 Consequence of thiourea on biochemical parameters

Many investigations have reported on effect of thiourea on biochemical parameters of different crops. Seed priming along with two foliar application of TU (1000 ppm) at growth stages of coriander plants with varying irrigation level showed significantly higher chlorophyll pigment (Shanu *et al.,* 2013). Similar observation was recorded by Amin *et al.* (2014) on faba bean with varying concentration of TU (250, 500 and 1000 mg L^{-1}) increased chlorophyll *a* and chlorophyll *b* on treated plants.

Foliar application of 500 ppm TU increased proline (2.41) content of salt stressed maize plants under salinity compared to untreated plants (1.04). TU altered the level of reactive oxygen species in plants under stress and promoted plant growth (Kaya *et al.,* 2015).

2.3.3.4 Consequence of thiourea on yield and quality attribute

Seed priming along with two foliar sprays of TU at 500 ppm concentration enhanced cowpea yield by 26 per cent (Anitha *et al.*, 2004).

Sahu and Singh, (1995) found that soil and foliar application of TU increased the yield components on wheat, soil applied TU at 10 kg/ha gave better grain yield (469 g/m^2) over control (399 g/m^2) likewise foliar application at flowering stage (0.5 kg/ha) gave good grain yield (406 g/m^2) over control (381 g/m^2).

Sahu *et al.* (1995) reported that an increase in grain yield (40.58%) in maize with application of TU at 1000 ppm by increasing harvest index at field condition.

According to Ravat and Makani, (2015) TU @ 500 ppm and 1000 ppm had good response on yield characters of okra. At 500 ppm it produced increased pod number (31.48%), average pod weight (7.7%), pod length (12.16%) and pod girth (3.3%) over untreated plants. The total seed yield were higher (122.3% and 37.63% respectively) from both treatments. Similarly foliar application of 1000 ppm TU three times on okra increased the yield character like maximum number of pods (21.7) in a single plant, pod length (5.7 cm) and diameter (15.5 mm) and total yield (15.3 ton) compared to non-sprayed plant yield (14.3, 12.2 cm, 12.6 mm,11.4 ton respectively).

Zain *et al.* (2017) claimed that TU @ 300 mg L^{-1} improved the productive tillers (316.22) and decreased unproductive tillers (12.11) of late sown wheat, also spike length (12.3 cm) and spikelets per spike (16.78) were more on the same treatment compared to the control (267.33, 17.11, 10.85 cm, 14.11 respectively). The number of grains per spike and total grain yield (24 %) were more with treatment.

Materials & Methods

3. MATERIALS AND METHODS

The investigation work entitled with "Physiological effect of seed priming and foliar application of growth regulators in cowpea (*Vigna unguiculata* L.)" was conducted in two experiments, including initial laboratory study followed by field study was conducted at College of Agriculture, Padannakkad and Instructional Farm II, Karuvacheri, College of Agriculture, Padannakkad. The objective of the study was elucidation of influence of seed priming and foliar application of growth regulators on morpho-physiological changes, yield and quality in cowpea (*Vigna unguiculata* L.). The particulars regarding materials and methods implemented for the study are described in this chapter.

Experiment 1: Effect of seed priming with growth regulators on germination and establishment of cowpea seeds.

Experiment 2: Field study on effect of seed priming and foliar application of growth regulators

3.1 SEED PRIMING WITH GROWTH REGULATORS

Laboratory study was conducted at College of Agriculture, Padannakkad, to understand pre sowing treatment effect of growth regulators at different concentration, on seed germination and seedling establishment of cowpea. Cowpea seed variety PGCP-6 was collected from Regional Agricultural Research Station, Pattambi, Palakkad. The growth regulators taken for the study are brassinolide, salicylic acid and thiourea, having eight treatments and three replications with 25 seeds in each replication. Seeds were soaked in respective solution for overnight.

3.1.1 Experiment details

Crop	: Cowpea
Vareity	: PGCP-6
Treatments	8

Replications 3

No of seeds per replication: 25

3.1.2 Treatment details

T_1: Control

T_2: Thiourea @ 500 ppm

T_3: Salicylic acid @ 50 ppm

T_4: Salicylic acid @ 100 ppm

T_5: Salicylic acid @ 150 ppm

T_6: Brassinolide @ 0.1 ppm

T_7: Brassinolide @ 0.3 ppm

T_8: Brassinolide @ 0.5 ppm

3.1.3 Preparation of seed priming solutions

Brassinolide: Brassinolide with specification assay minimum 90 per cent purity manufactured by Maharashtra based Sisco Research Laboratories private limited, was used for preparing the solution. Initially 100 ppm stock solution was prepared by dissolving 10 mg brassinolide in few drops of ethanol and made 100 ml with distilled water, later from the stock 10 ppm solution was prepared by taking 10 ml of stock solution and made to 100 ml. From this 10-ppm solution 0.1, 0.3 and 0.5 ppm solutions were prepared.

Salicylic acid: The compound salicylic acid with 99 per cent purity was used for preparing the solution. The chemical manufactured by Kochi based company namely, Spectrum Reagent and Chemicals, Pvt Ltd. Initially a stock solution was prepared with concentration of 200 ppm by dissolving 200 mg chemical in few drops of ethanoland made it to 1000 ml with distilled water. From this stock 50, 100 and 150 ppm solutions were prepared.

Thiourea: Thiourea with 99 per cent purity used for the study. It was manufactured by Isochem Laboratories, Kochi, 500 ppm solution was prepared by dissolving 500 mg thiourea in 1000 ml distilled water.

3.1.4 Observations on seed germination and early seedling growth

Overnight primed seeds were placed in moist tissue paper kept in petri plate, later transferred to protray filled with coir pith compost. Observations recorded up to 10 DAS.

3.1.4.1 Number of days from sowing to seed germination

Days taken for seed germination counted in daily intervals.

3.1.4.2 Percentage of sprouting

Sprouting percentage noted by counting seed with sprouts and its per cent calculated from total number of seeds kept for study.

3.1.4.3 Days taken for 50 % germination

Days taken for seeds to germinate and become 50 % germination were counted in daily intervals.

3.1.4.4 Germination percentage

Germination percentage calculated on 10^{th} DAS in protray.

3.1.4.5 Percentage of survival

Percentage of survival calculated by excluding damaged seedlings.

3.1.4.6 Whole seedling weight

Whole seedling dry weight was calculated after keeping selected seedling in hot air oven at 60°C for 48 hrs.

3.1.4.7 Seedling vigour index

Seedling vigour index was calculated by multiplying germination percentage with whole seedling dry weight (Abdul-Baki and Anderson, 1973).

3.2 FIELD EXPERIMENT

The Experiment 2 was carried out under field conditions using the best seed priming results obtained from Experiment 1. The study aimed to elucidate the combination effect seed priming and foliar application of growth regulators on cowpea. The experimental plot was selected at Instrumental Farm II, Karuvacheri, College of Agriculture, Padannakkad, during the period of January to May 2021. Experiment was carried out in randomized block design including 14 treatments and three replications, with 50 cowpea plants in each plot. Both best primed seed and control seeds chosen for sowing and foliar sprays were given with different concentrations of brassinolide, salicylic acid and thiourea at 20 and 40 days after sowing. Initial land operations, manuring, fertilizer application, and cultural practices were followed based on POP, KAU (2016).

3.2.1 Location and weather condition of experimental area

The experimental plot was located in 4th block of Instructional Farm II, Karuvacheri, College of Agriculture, Padannakkad, located at $12^0$14'45''N latitude and $75^0$8'6'' E longitude at an elevation of 9 m above mean sea level. Data on various weather parameters during crop period were collected from the Agromet Observatory of RARS, Pilicode and depicted graphically in Fig. 1 and summarized in Appendix I.

3.2.2 Experimental site

The experimental plot having well drained red loamy soil, ragi was cultivated previously in the field area.

3.2.3 Design and layout of the experiment

Season	: January- May 2021
Variety	: PGCP – 6
Spacing	: 30 cm × 15 cm
Plot size	: 1.5 m ×1.5 m
Design	: RBD
Treatments	14

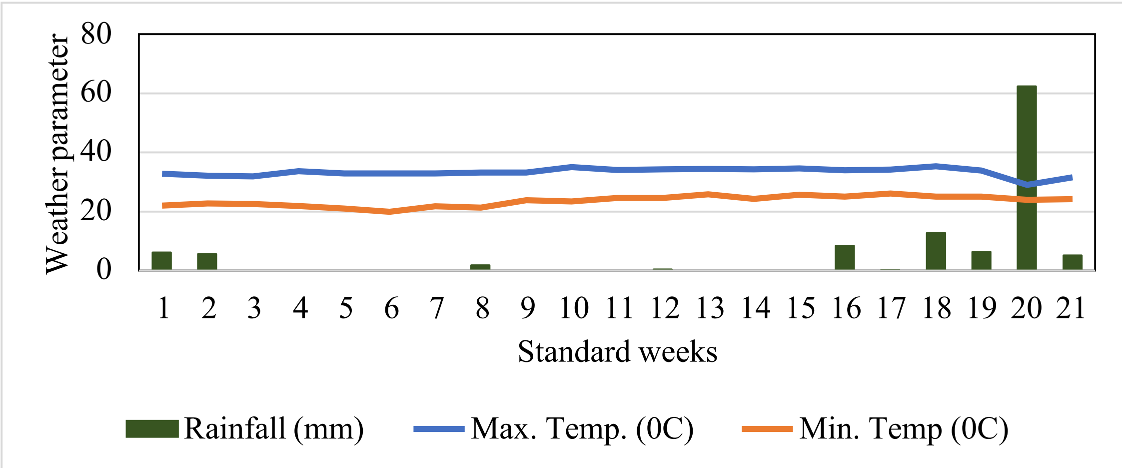
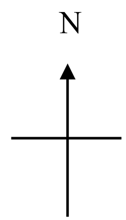


Figure 1: Weather parameters in standard weeks during the crop season.



R_1T_{10}	R_1T_4	R_1T_2	R_1T_5	R_1T_8	R_1T_{12}	R_1T_7	R_1T_9	R_1T_6	R_1T_{13}	R_1T_1	R_1T_{11}	R_1T_{14}	R_1T_3
R_2T_6	R_2T_{13}	R_2T_{10}	R_2T_3	R_2T_5	R_2T_8	R_2T_{11}	R_2T_2	R_2T_{12}	R_2T_7	R_2T_4	R_2T_9	R_2T_1	R_2T_{14}
R_3T_{12}	R_3T_5	R_3T_3	R_3T_6	R_3T_{14}	R_3T_1	R_3T_4	R_3T_{13}	R_3T_9	R_3T_{11}	R_3T_2	R_3T_7	R_3T_{10}	R_3T_8

Figure 2. Layout of the experimental plot

- **Treatment details**

T_1: Standard POP without TU (KAU, 2016)

T_2: Standard POP with TU @ 500 ppm FS

T_3: T_1 + SA @ 50 ppm FS

T_4: SA @ 100 ppm SP + SA @ 50 ppm FS

T_5: T_1 + SA @ 100 ppm FS

T_6: SA @ 100 ppm SP + SA @ 100 ppm FS

T_7: T_1 + SA @ 150 ppm FS

T_8: SA @ 100 ppm SP + SA @ 150 ppm FS

T_9: T_1 + BR @ 0.1 ppm FS

T_{10}: BR @ 0.3 ppm SP + BR @ 0.1 ppm FS

T_{11}: T_1 + BR @ 0.3 ppm FS

T_{12}: BR @ 0.3 ppm SP + BR @ 0.3 ppm FS

T_{13}: T_1+ BR @ 0.5 ppm FS

T_{14}: BR @ 0.3 ppm SP + BR @ 0.5 ppm FS

Plot size: 1.5 m ×1.5 m

Spacing: 30 cm × 15 cm

Design : RBD

SA- Salicylic acid

BR- Brassinolide

TU- Thiourea

SP- Seed priming

FS- Foliar spray

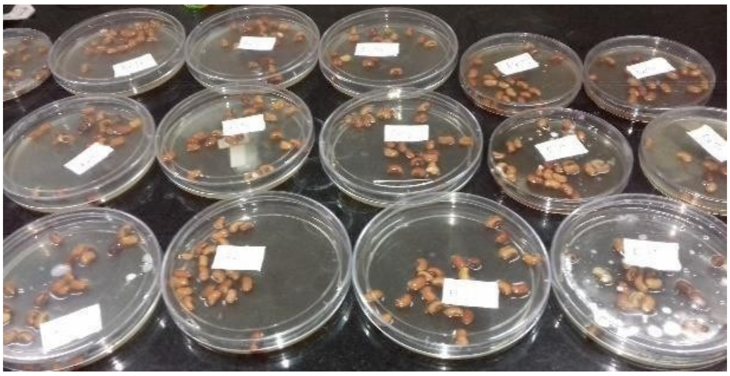


Plate 1 A: Seed priming with growth regulators

Plate 1 B: Sprouts in moist tissue paper

Plate 1 C: Sprouts transferd into potray

Plate 1 D: Seedlings at 10 DAS

Plate 1: Seed priming and early seedling study, College of Agriculture, Padannakkad

Plate 2. Initial land preparation

Plate 3. Dolomite application

Plate 4. Seed priming with growth regulators and control in water

Plate 5. Sowing of seeds

Plate 6. Seedling emergence

Replications 3

No. of plants per plot : 50

Date of sowing : 15^{th} January 2021

3.2.4 Treatment details

T_1: Standard POP without Thiourea (KAU, 2016).

T_2: Standard POP with 500 ppm Thiourea

T_3: T_1 + Salicylic acid @ 50 ppm

T_4: Best pre-treated seed + Salicylic acid @ 50 ppm

T_5: T_1 + Salicylic acid @ 100 ppm

T_6: Best pre-treated seed + Salicylic acid @ 100 ppm

T_7: T_1 + Salicylic acid @ 150 ppm

T_8: Best pre-treated seed + Salicylic acid @ 150 ppm

T_9: T_1 + Brassinolide spray @ 0.1 ppm

T_{10}: Best pre-treated seed + Brassinolide spray @ 0.1 ppm

T_{11}: T_1 + Brassinolide spray @ 0.3 ppm

T_{12}: Best pre-treated seed + Brassinolide spray @ 0.3 ppm

T_{13}: T_1 + Brassinolide spray @ 0.5 ppm

T_{14}: Best pre-treated seed + Brassinolide spray @ 0.5 ppm

Foliar spray was given at 20 and 40 DAS during early morning hours and observations taken at 25, 45 and 60 DAS.

3.3 FIELD PREPARATION

Stepwise land operations from initial land ploughing with tractor, levelling, bed preparations are carried out accordingly.

3.3.1 Land preparation, fertilizer application and sowing

Prior to land operations, soil samples were collected randomly from five spots of field at a depth of 15 cm, later all samples mixed uniformly. It used for analysis of pH, EC, micro and macro nutrient status of soil. The experimental plot thoroughly ploughed with mould board plough and tractor, removed weeds and stubbles ofprevious crops and made the field fine tilth, then the plot irrigated uniformly.

The levelled land made into three blocks in length, those blocks again made to 14 subplots. Totally 42 beds each with 1.5 x1.5 m² dimension is represented in layout (Fig. 2). In each plots dolomite were applied to correct pH @ 400 Kg/ ha, after one week the basal dose of Farm yard manure applied @ 20 t/ha. Basal dose of N:P: K were given as per recommendation.

Methods followed for soil analysis

Sl No.	Parameters	Methods	Reference
1	pH	Potentiometric method	Jackson (1958)
2	EC	Conductivity meter	Jackson (1958)
3	Organic carbon	Wet digestion method	Walkley and Black (1934)
4	Available nitrogen	Alkaline permanganate method	Subbiah and Asija (1956)
5	Available phosphorous	Bray-1 extract with colorimetry	Jackson (1958)
6	Available potassium	Neutral normal ammonium acetate with flame photometer	Jackson (1958)
7	Available calcium	Atomic absorption spectrophotometer	Jackson (1958)
8	Available magnesium	Atomic absorption spectrophotometer	Jackson (1958)
9	Available sulphur	Turbidimetrically using spectrophotometer	Massouni and Cornfield (1963)
10	Available copper	Atomic absorption spectrophotometer	Emmel *et al.* (1977)
11	Available iron	Atomic absorption spectrophotometer	Sims and Johnson (1991)
12	Available zinc	Atomic absorption spectrophotometer	Emmel *et al.* (1977)
13	Available manganese	Atomic absorption spectrophotometer	Sims and Johnson (1991)
14	Available boron	Hot water extraction and spectrophotometer estimation	Binghum (1982)

3.3.2 Seed priming and sowing

As per the results obtained from Experiment 1, overnight seed priming in solutions and control seeds soaked in distilled water. Later it subjected to drying to its original volume under shade. Seeds were sown after slight irrigation at spacing of 30 x 15 cm² with two seeds per hill.

3.3.3 Irrigation and after cultivation

After cultivation practices were followed timely as per KAU (2016) POP recommendations. Slight irrigation given next day after sowing. Need based intercultural operation including thinning, weeding and hoeing were given and soil made adequate aeration using spade and fork. Second dose of nitrogen given at 15 DAS.

3.3.4 Plant protection measures

Stem fly attack was noticed at initial seedling stage, it was more in control plant than treated plants. It was controlled with Acephate (2g/L) spray. At pod filling stage incidence of pod borer was noticed and it controlled with flubendiamide (20 %) @ 3g/L spray.

3.3.5 Harvesting of mature pods

Matured pods harvested manually. Separate harvesting carried out from each treatment.

3.4 OBSERVATIONS

Morphological, Physiological and biochemical observations were carried out at 25, 45 and 60 DAS. Five representative plants were selected randomly from each plot and tagged permanently using coloured ribbons for taking all observations throughout the experimental study period.

3.4.1 Morphological and phenological observations

3.4.1.1 Plant height

Height of representative plants measured using meter scale from base of the plant to the tip of main stem and the average value recorded in centimetres.

3.4.1.2 Leaf area per plant

Freshly harvested leaves from representative plants were used for measuring leaf area per plant. It was measured using portable leaf area meter.

3.4.1.3 Number of compound leaves per plant

Total number of fully opened matured trifoliate leaves of representative plants were counted.

3.4.1.4 Days to 50 per cent flowering

Days taken to get 50 per cent flowering were counted from the day of sowing to 50 per cent flowering of plots.

3.4.1.5 Days to first harvest

Days to first harvest counted from the day of sowing separately in each plot.

3.4.1.6 Duration of the crop

Duration of crop recorded in days from sowing to its final harvest.

3.4.1.7 Pod length & diameter

Ten pods from representative plants were randomly selected, pod length and diameter measured using thread and meter scale in centimetre and the average value was calculated.

3.4.1.8 Occurrence of pest and diseases

Pest and disease incidence observed separately in each treatment.

3.4.2 Physiological observations

3.4.2.1 Leaf area index

Leaf area index calculated by dividing total leaf area with ground crop area on which the plants grow. Ground area obtained from plant spacing. It is a unitless parameter.

$$LAI = \frac{\text{Leaf area}}{\text{Ground area}}$$

3.4.2.2 Relative growth rate

According to Blackman, (1919) RGR is the increase in dry weight at a particular time per unit material existing. It is expressed in mg g^{-1} d^{-1}.

$$RGR = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

W_1 = Dry weight of plant at the time of T_1

W2= Dry weight of plant at the time of T_2

3.4.2.3 Total dry matter production (g)

The whole plants selected randomly and uprooted without breaking the root system. It subjected to initial shade drying later cut into separate parts and kept in hot air oven for 48 hr at 60^0 C till it recorded a constant dry weight. The mean value of total dry matter production was calculated and expressed in gram (g).

3.4.2.4 SPAD chlorophyll meter readings (SCMR)

Chlorophyll reading recorded from representative plants at morning hour's using SCMR (Model-502 Konica Minolta, Japan)

3.4.2.5 Relative water content

Fresh leaf samples collected made into small bits and taken the fresh weight separately from each treatment, later the bits kept in distilled water for two hours. After swiped the excess water from leaf surfaces using blotting paper and taken the turgid weight. Then the samples kept in hot air oven for 48 hr at 60^0 C. RWC calculated using Barrs and Weatherly (1962) equation. It is expressed in per cent.

$$\text{Relative water content (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

3.4.2.6 Crop growth rate

It specifies change in dry matter accumulation over a period of time. CGR expressed in g m^{-2} day^{-1}.

$$CGR = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{1}{P}$$

W_1 = Dry weight of plant in gram at time T_1

W_2 = Dry weight of plant in gram at time T_2

P = Unit crop area

3.4.2.7 Specific leaf weight

It is the ratio between total leaf dry weight and leaf area. It specifies relative thickness of leaf and it expressed in mg cm^{-2}.

$$SLW = \frac{\text{leaf weight}}{\text{leaf area}}$$

3.4.2.8 Fv/Fm, Y(II) and ETR

Ratio of chlorophyll fluorescence, quantum yield and electron transport rate were measured from representative plant leaves during morning hours using stress kit (ADC biosynthetic Ltd, Glodal house, Gedding Road, London).

3.4.3 Biochemical observations

Biochemical observation of plant samples carried out in laboratory at 25, 45 and 60 DAS.

3.4.3.1 Chlorophyll content

Freshly harvested leaf samples were taken for chlorophyll estimation. The samples made small bit pieces and 250 g sample were incubated in 20 ml Dimethyl sulfoxide overnight. The chlorophyll pigment was extracted into the solvent. Later with the help of UV-Spectrophotometer absorbance values noted at wavelength of 663, 645, 510 and 480 nm and chlorophyll *a*, chlorophyll *b* and total chlorophyll content calculated using formulae specified by Hiscox and Israel, (1979). It expressed in mg g^{-1}.

Chlorophyll a = [(12.7xA 663) - (2.69xA 645)] x V/1000xW

Chlorophyll b = [(22.9xA 645) - (4.68xA 663)] x V/1000xW

Total chlorophyll = [(20.2xA 645) + (8.02xA 663)] x V/1000xW

Where,

A - Absorption at given wavelength

V - Volume of supernatant solution

W- Weight of the sample

3.4.3.2 Proline content (µg g-1)

Proline content of randomly selected leaf samples analysed in a series of steps with specified chemicals according to Bates et al. (1973). Using mortar and pestle 0.5 g fresh leaf sample macerated with 10 ml of sulfosalicylic acid (3%). 2 ml filtrate taken to a test tube, to which 2 ml glacial acetic acid and freshly prepared acid ninhydrin were added then kept the test tubes in boiling water bath for 1 hr. After the time period kept in ice bath, later 4 ml toluene added and subjected to uniform mixingusing vortex. Clear transparent portion of the sample separated out using separating funnel and OD value of this portion recorded at 520 nm with spectrophotometer.

$$\mu g \text{ moles/ g tissue proline} = \frac{\mu g \text{ proline ml x volume of toluene}}{115.5} \times \frac{5}{\text{weight of sample}}$$

3.4.4 Yield and pod quality observations

Observation of yield and quality parameters were taken after drying of matured pods.

3.4.4.1 Average number of pods per plant

Mean value of total number of pods from five index plants counted and recorded till the harvest.

3.4.4.2 Average number of seeds per pod

From ten pods of selected five plants total number of seeds counted and recorded the mean value.

3.4.4.3 Average number of seeds per plant

Randomly selected five plants and collected whole pods and counted the total number of seeds in each plant separately and the average value were noted.

3.4.4.4 Weight of 100 seeds

100 seeds counted from each treatment and weighted separately, noted in gram.

3.4.4.5 Seed yield/plant/plot/ha

Total seed yield from each treatment was noted in per plant, per plot and per hectare.

3.4.5 Economics

3.4.5.1 Net returns

Cost of cultivation of entire experiments calculated and net returns worked out based on market price of grain. Net return worked out using formula as

Net income = Gross income -Total expenditure

3.4.5.2. Benefit-cost ratio (BCR)

Benefit-cost ratio of the experimental study worked out using formula:

$$\text{Benefit: Cost} = \frac{\text{Gross income}}{\text{Cost of cultivation}}$$

3.5 DATA ANALYSIS

The data obtained from both experiments analysed separately. Observations of Experiment 1 studied and analysed using Completely randomized design and Experiment 2 was done in Randomized block design. Significance checked in each observations using F test (Snedecor and Cochran, 1967). Critical difference worked out at 5 per cent level when F value were significant. All the statistical analysis were carried out using statistical package GRAPES 1.0.0. It was used for interpretation and comparing the results.

Plate 7. Field at 20 DAS

Plate 8. Field at 30 DAS

Plate 9. Field at 40 DAS

Plate 10. Field at 55 DAS

Plate 11. Flowering stage

Plate 12. Pod formation

Plate 13. Harvesting of mature pods

Plate 14. Drying of harvested pods

Plate 15. Foliar spray of growth regulators

Plate 16. Recording of observations

Plate 17. General view of experimental plot

Plate 18. Stem fly incidence

Plate 19. Pod borer attack

Results

3. RESULTS

The research work entitled "Physiological effect of seed priming and foliar application of growth regulators in cowpea (*Vigna unguiculata* L.)" was completed with two experiments. Experiment 1 was conducted as early seedling growth studies at College of Agriculture, Padannakkad. Experiment 2 was the field study done at Instructional Farm II, Karuvacheri, College of Agriculture, Padannakkad. The study was to examine the effect of seed priming and foliar spray of various growth regulators viz. brassinosteroid, salicylic acid and thiourea on cowpea seed germination, early seedling vigour, growth and yield. The observations noted from theexperiments were analysed statistically and results are presented in Table forms in thischapter.

Experiment 1

4.1 Studies on seed germination and early seedling growth

The data on seed germination and early seedling growth parameters are presented in Table 1. The minimum number of days taken for seed germination in T_4, T_6 and T_7 with four days. The maximum number of days from sowing to seed germination was recorded in T_3 (5.67) which was on par with T_8 (5.33). 100 per cent seed sprouting recorded in T_4 and T_7. Minimum number of days taken for 50 per cent seed germination was in treatment T_7 (1.33) followed by T_4 and T_6 with same number of two days. Germination per cent had no significant difference among all the treatments. Although not significant, 100 per cent seed germination was recorded in treatments T_4 and T_7 and lowest in control (93.33 per cent). 100 per cent of seedling survival was observed in treatment T_4 followed by T_7 (98.67 per cent). The highest whole seedling dry weight was obtained in T_7 (0.36 g) followed by T_6 (0.32 g) and T_8 (0.31 g). T_7 (36.67) recorded significantly higher seedling vigour index followed byT_4 (31.67). The lowest seedling vigour index was noted in control (23.95). The seed priming in 100 ppm salicylic acid and 0.3 ppm brassinolide were found as best seed priming treatments. These results were further evaluated under field conditions.

Table 1: Effect of seed priming with various growth regulators on seed germination and early seedling growth

Treatments	Days to seed germination (days)	Percentage of sprouting (%)	50 % seed germination (days)	Seed germination percentage (%)	Percentage of seedling survival (%)	Whole seedling dry weight (g)	Seedling vigour index (10 DAS)
T_1: Control	4.67^{c}	94.67^{c}	2.67^{ab}	93.33	92.00^{c}	0.25^{d}	23.95^{e}
T_2: TU @ 500 ppm	5.00^{bc}	96.67^{bc}	3.33^{a}	97.33	93.33^{bc}	0.28^{cd}	26.63^{cde}
T_3: SA @ 50 ppm	5.67^{a}	97.33^{b}	3.67^{a}	96.00	93.33^{bc}	0.26^{d}	24.63^{de}
T_4: SA @ 100 ppm	4.00^{d}	100.00^{a}	2.00^{bc}	100.00	100.00^{a}	0.32^{b}	31.67^{b}
T_5: SA @ 150 ppm	5.00^{bc}	97.33^{b}	3.33^{a}	97.33	97.33^{ab}	0.29^{bcd}	27.59^{bcde}
T_6: BR @ 0.1 ppm	4.00^{d}	96.67^{bc}	2.00^{bc}	97.33	96.00^{abc}	0.31^{b}	30.13^{bc}
T_7: BR @ 0.3 ppm	4.00^{d}	100.00^{a}	1.33^{c}	100.00	98.67^{a}	0.36^{a}	36.67^{a}
T_8: BR @ 0.5 ppm	5.33^{ab}	95.67^{bc}	2.67^{ab}	96.00	93.33^{bc}	0.30^{bc}	28.32^{bcd}
SE(m)	0.204	0.791	0.354	1.70	1.76	0.01	1.41
CD (0.05)	0.612	2.370	1.06	NS	5.29	0.004	4.23

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

Before starting the field experiment, soil nutrient status was analysed and the data presented in Table 2.

Table 2: Soil test report before the experiment

Sl. No.	Parameters	Quality	Remarks
1	pH	5.10	Strongly acidic
2	Electrical conductivity (dS m^{-1})	0.04	Normal
3	Organic carbon (%)	0.42	Low
4	Available nitrogen (kg ha^{-1})	156.00	Low
5	Available phosphorous (kg ha^{-1})	206.40	High
6	Available potassium (kg ha^{-1})	215.22	Medium
7	Available calcium (mg kg^{-1})	140.90	Deficient
8	Available magnesium (mg kg^{-1})	11.02	Deficient
9	Available sulphur (kg ha^{-1})	1.30	Deficient
	Micronutrients		
10	Copper (mg kg^{-1})	1.05	Deficient
11	Iron (mg kg^{-1})	6.68	Deficient
12	Zinc (mg kg^{-1})	1.52	Deficient
13	Manganese (mg kg^{-1})	25.00	Sufficient
14	Boron (mg kg^{-1})	0.193	Deficient

The experimental plot was strongly acidic with low organic carbon and available nitrogen. Soil contains high phosphorus and medium potassium. Deficiency of available calcium, magnesium, sulphur, copper, zinc and boron were noted. Dolomite @ 400 kg ha^{-1} was applied based on KAU POP recommendation during land preparation. FYM was applied @ 20 t ha^{-1} one week after dolomite application and mixed thoroughly with soil and need based irrigations were given accordingly. Nitrogen, phosphorous and potassium fertilizers were applied as per recommendation.

4.2 MORPHOLOGICAL AND PHENOLOGICAL OBSERVATIONS

4.2.1 Effect on plant height

Table 3: Effect of various growth regulators on plant height at various growth stages of cowpea.

Treatments	Mean plant height (cm)		
	25 DAS	**45 DAS**	**60 DAS**
T_1: Standard POP without TU (KAU, 2016)	17.38^{i}	28.87^{f}	35.71^{f}
T_2: Standard POP with TU foliar spray @ 500 ppm	19.27^{gh}	32.27^{e}	37.41^{de}
T_3: T_1 + SA foliar spray @ 50 ppm	18.91^{h}	32.31^{e}	37.31^{de}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	20.14^{cde}	32.69^{cde}	37.7^{cde}
T_5: T_1 + SA foliar spray @ 100 ppm	19.67^{f}	32.45^{de}	37.35^{de}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	21.09^{b}	34.04^{a}	40.13^{a}
T_7: T_1 + SA foliar spray @ 150 ppm	19.58^{fg}	32.38^{e}	37.13^{e}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	20.29^{cd}	33.73^{bcd}	38.15^{bc}
T_9: T_1 + BR foliar spray @ 0.1 ppm	19.31^{g}	32.51^{de}	37.27^{de}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1ppm	19.91^{cde}	33.93^{bc}	37.91^{bcd}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	19.78^{ef}	32.57^{de}	37.53^{cde}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	20.35^{c}	33.95^{bc}	38.47^{b}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	19.94^{def}	32.99^{bcde}	37.57^{cde}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	21.73^{a}	35.71^{a}	40.64^{a}
SE(m)	0.124	0.456	0.232
CD (0.05)	0.361	1.33	0.675

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

The data on effect of various growth regulators on mean plant height was recorded at 25, 45 and 60 DAS and presented in Table 3. The results confounded that plant height increased when treated with various growth regulators compared to control plants. At 25 DAS, the plant height was maximum when it was subjected to seed priming and foliar spray of BR in T_{14} (21.73 cm) followed by T_6 (21.09 cm). Lowest mean plant height recorded in control T_1 (17.38 cm).

At 45 DAS, the maximum plant height was recorded in treatment T_{14} (35.71 cm) and T_6 (34.04 cm). The lowest value was noted in T_1 (28.87 cm).

At 60 DAS, maximum plant height was observed in treatment T_{14} (40.64 cm) and T_6 (40.13 cm) followed by T_{12} (38.47 cm) which were on par with T_8 (38.15 cm) and T_{10} (37.91 cm). The lowest plant height was documented in treatment T_1 (35.71 cm). With foliar spray of thiourea plant height increased by 10.87, 11.78 and 4.8 per cent over control plants at 25, 45 and 60 DAS, respectively.

4.2.2 Effect on leaf area per plant

Table 4: Effect of various growth regulators on leaf area per plant (cm^2) at various growth stages of cowpea.

Treatments	Mean leaf area per plant (cm^2)		
	25 DAS	45 DAS	60 DAS
T_1: Standard POP without TU (KAU, 2016)	163.49^{g}	388.21^{i}	558.93^{f}
T_2: Standard POP with TU foliar spray @ 500 ppm	181.29^{d}	481.50^{efgh}	689.93^{bcd}
T_3: T_1 + SA foliar spray @ 50 ppm	173.55^{f}	474.83^{fgh}	631.61^{e}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	181.65^{d}	489.43^{defg}	689.96^{bcd}
T_5: T_1 + SA foliar spray @ 100 ppm	180.45^{de}	489.38^{defg}	685.22^{cd}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	204.97^{b}	528.72^{ab}	735.95^{a}
T_7: T_1 + SA foliar spray @ 150 ppm	176.56^{ef}	483.80^{efgh}	677.15^{d}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	181.43^{d}	504.05^{cb}	693.61^{bc}
T_9: T_1 + BR foliar spray @ 0.1 ppm	181.15^{d}	469.37^{h}	675.37^{d}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	183.89^{cd}	493.88^{de}	695.07^{bc}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	181.92^{cd}	473.36^{gh}	686.85^{cd}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	186.22^{c}	516.11^{cd}	703.95^{b}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	183.03^{cd}	492.21^{def}	688.04^{cd}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	210.76^{a}	541.37^{a}	734.93^{a}
SE(m)	1.49	6.33	5.37
CD (0.05)	4.32	18.42	15.60

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

The data on effect of various growth regulators on mean leaf area per plant was recorded 25, 45 and 60 DAS and presented in Table 4. At 25 DAS, maximumleaf area per plant recorded in treatment T_{14} (210.46 cm^2) followed by T_6 (204.97 cm^2) and T_{12} (186.22 cm^2). T_{12} was on par with T_{13} (183.03 cm^2), T_{10} (183.89 cm^2) and T_{11} (181.92 cm^2). T_2 (181.29 cm^2), T_4 (181.65 cm^2), T_8 (181.43 cm^2) and T_9 (181.15 cm^2) were on par each other. T_1 (163.49) recorded the lowest leaf area per plant.

At 45 DAS, maximum leaf area per plant was recorded in treatment T_{14} (541.37 cm^2) which was on par with T_6 (528.72 cm^2). Least leaf area was recorded in the treatment T_1 (388.21 cm^2).

At 60 DAS, the leaf area per plant was maximum in treatment T_{14} (734.93 cm^2) and T_6 (735.95 cm^2) followed by T_{12} (703.95 cm^2) which was on par with T_{10} (695.07 cm^2), T_8 (693.61 cm^2), T_4 (689.96 cm^2) and T_2 (689.93 cm^2). Lowest leaf area was recorded in treatment T_1 (558.93 cm^2).

4.2.3 Effect on number of compound leaves per plant

The data on effect of various growth regulators on mean number of compound leaves per plant was recorded 25, 45 and 60 DAS and presented in Table 5. Maximum number of compound leaves was observed at 25 DAS in treatment T_6 (5.27) and in T_{14} (5.27), followed by T_{12} (4.80), T_8 (4.73), T_{10} (4.73) and T_4 (4.67). Least number of compound leaves was recorded in T_1 (4.07). At 45 DAS, there was no significant difference among all treatments. Number of compound leaves per plant at 60 DAS had shown significant difference. T_6 (26.80) and T_{14} (26.47) showed best response of number of leaves per plant followed by T_{12} (25.00) which was on par with T_5 (23.80), T_8 (24.47), T_{10} (24.40) and T_{13} (24.20). The least number of compound leave per plant was observed in control plants (17.07).

Table 5: Effect of growth regulators on mean number of compound leaves per plant at various growth stages of cowpea.

Treatments	Average number of compounds leaves per plant		
	25 DAS	45 DAS	60 DAS
T_1: Standard POP without TU (KAU, 2016)	4.07^{f}	14.60	17.07^{f}
T_2: Standard POP with TU foliar spray @ 500 ppm	4.40^{cd}	16.33	22.13^{e}
T_3: T_1 + SA foliar spray @ 50 ppm	4.20^{ef}	16.13	18.27^{f}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	4.67^{b}	16.73	23.47^{cde}
T_5: T_1 + SA foliar spray @ 100 ppm	4.47^{c}	17.20	23.80^{bcd}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	5.27^{a}	19.93	26.80^{a}
T_7: T_1 + SA foliar spray @ 150 ppm	4.40^{cd}	17.20	22.13^{e}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	4.73^{b}	17.67	24.47^{bc}
T_9: T_1 + BR foliar spray @ 0.1 ppm	4.27^{de}	16.67	22.80^{de}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	4.73^{b}	17.47	24.40^{bc}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	4.27^{de}	17.07	23.20^{cde}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	4.80^{b}	18.40	25.00^{b}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	4.40^{cd}	17.27	24.20^{bcd}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	5.27^{a}	19.67	26.47^{a}
SE(m)	0.063	1.418	0.489
CD (0.05)	0.184	NS	1.42

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

4.2.4 Effect on days to 50 per cent flowering, first harvest and duration of crop

The data on effect of various growth regulators on mean number of days taken to 50 per cent flowering and days to first harvest was recorded and presented in Table 6. Observations of number of days taken for 50 per cent flowering showed significant difference among all treatments. T_{14} (33.22) took the least number of days among all the treatments for 50 % flowering. T_1 (51.00) took maximum number of days for 50 per cent flowering.

The least number of days to first harvest was recorded in treatment T_6 (52.00) and T_{14} (53.67). More number of days for first harvest was noted in control (66.67) which was on par with T_9 (65.33) and T_3 (65.00) followed by T_2 (64.33) and T_7 (63.67).

Duration of crop was highest in treatment T_{14} (85.67) and T_6 (85.33) which were on par with T_{12} (83.67) followed by T_5, T_8 and T_{10} (80.67). Minimum crop duration recorded in control plants (74.67) which was on par with T_3 (76.33) and T_9 (75.33).

Table 6: Effect of various growth regulators on days to 50 per cent flowering, days to first harvest and duration of cowpea.

Treatments	Days to 50 % flowering	Days to first harvest	Duration of crop
T_1: Standard POP without TU (KAU, 2016)	51.00[a]	66.67[a]	74.67[f]
T_2: Standard POP with TU foliar spray @ 500 ppm	48.33[bc]	64.33[b]	77.67[d]
T_3: T_1 + SA foliar spray @ 50 ppm	48.67[bc]	65.00[ab]	76.33[def]
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	47.00[c]	58.00[d]	78.00[d]
T_5: T_1 + SA foliar spray @ 100 ppm	48.00[bc]	59.00[d]	80.67[c]
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	34.00[f]	52.00[g]	85.33[a]
T_7: T_1 + SA foliar spray @ 150 ppm	47.00[c]	63.67[b]	77.33[de]
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	44.67[d]	55.33[ef]	80.67[c]
T_9: T_1 + BR foliar spray @ 0.1 ppm	47.00[c]	65.33[ab]	75.33[ef]
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	43.00[de]	58.00[d]	80.67[c]
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	49.00[b]	61.00[c]	77.33[de]
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	42.00[e]	55.67[e]	83.67[ab]
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	47.33[bc]	61.00[c]	82.0[bc]
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	33.22[f]	53.67[fg]	85.67[a]
SE(m)	0.592	0.65	0.743
CD (0.05)	1.72	1.89	2.16

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

4.2.5 Effect on pod length and pod diameter

Table 7: Effect of growth regulators on pod length and diameter of cowpea.

Treatments	Pod length (cm)	Pod diameter (cm)
T_1: Standard POP without TU (KAU, 2016)	16.83[i]	1.13[e]
T_2: Standard POP with TU foliar spray @ 500 ppm	17.44[fg]	1.14[de]
T_3: T_1 + SA foliar spray @ 50 ppm	17.27[h]	1.14[cde]
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	17.54[de]	1.16[bcde]
T_5: T_1 + SA foliar spray @ 100 ppm	17.48[ef]	1.18[abc]
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	18.21[a]	1.22[a]
T_7: T_1 + SA foliar spray @ 150 ppm	17.38[g]	1.17[bcde]
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	17.88[b]	1.18[abcd]
T_9: T_1 + BR foliar spray @ 0.1 ppm	17.48[ef]	1.15[bcde]
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	17.83[bc]	1.17[bcde]
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	17.59[d]	1.16[bcde]
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	17.87[bc]	1.17[bcde]
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	17.78[c]	1.17[bcde]
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	18.21[a]	1.19[ab]
SE(m)	0.031	0.015
CD (0.05)	0.090	0.045

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

The data on effect of various growth regulators on pod length and pod diameter was recorded and presented in Table 7. Maximum pod length was recordedin treatment T_6 and T_{14} (18.21) followed by T_8 (17.88) which was on par with T_{10} (17.83) and T_{12} (17.87). Minimum pod length was noted in treatment T_1 (16.83) among all the treatments.

More pod diameter was recorded in treatments T_6 (1.22) which was on par with T_{14} (1.19), T_5 (1.18) and T_8 (1.18). T_2 (1.14) recorded the least pod diameter among all the treatments.

4.2.6 Effect on occurrence of pest and diseases

Pest incidence observed in cowpea during crop growing period were stem fly (*Ophiomyia phaseoli*) during the initial seedling stage at lower stem portion. Stem fly incidence was higher in control plant. Salicylic acid treated plant showed less incidence over control. Pod borer (*Maruca vitrata*) incidence was noted at pod filling stage. Pest incidence was more in control plants in comparison with all treated plants. Acephate 2 g L^{-1} used for management of stem fly. Pod borer managed with flubendamide 3 g L^{-1} spray.

4.3 PHYSIOLOGICAL OBSERVATIONS

4.3.1 Effect on Leaf Area Index (LAI)

The data on effect of various growth regulators on LAI was recorded at 25, 45 and 60 DAS and presented in Table 8. At 25 DAS, the highest LAI was recorded in treatment T_{14} (0.46) and T_6 (0.45) followed by T_2 (0.41), T_8 (0.41), T_{10} (0.41), T_{12} (0.41) and T_{13} (0.41) which was on par with T_5 (0.40) and T_{11} (0.40). Minimum index recorded in treatment T_1 (0.36).

At 45 DAS, highest LAI was in treatment T_{14} (1.20) and T_6 (1.18) followed by T_8 (1.11) and T_{12} (1.11) which was on par with T_{10} (1.10) and T_{13} (1.10). Least LAI was recorded from control plants (0.86).

At 60 DAS, the highest LAI was observed in treatment T_6 (1.64) and T_{14} (1.63) followed by T_{12} (1.56). The smallest LAI was showed in treatment T_1 (1.25).

Table 8: Effect of various growth regulators on LAI at various growth stages of cowpea.

Treatments	Leaf area index		
	25 DAS	45 DAS	60 DAS
T_1: Standard POP without TU (KAU, 2016)	0.36^{f}	0.86^{f}	1.25^{h}
T_2: Standard POP with TU foliar spray @ 500 ppm	0.41^{bc}	1.07^{cde}	1.51^{cdef}
T_3: T_1 + SA foliar spray @ 50 ppm	0.38^{e}	1.05^{de}	1.46^{g}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	0.40^{cd}	1.09^{bcd}	1.53^{bcde}
T_5: T_1 + SA foliar spray @ 100 ppm	0.40^{bcd}	1.08^{bcde}	1.50^{def}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	0.45^{a}	1.18^{a}	1.64^{a}
T_7: T_1 + SA foliar spray @ 150 ppm	0.39^{de}	1.07^{bcde}	1.49^{fg}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	0.41^{bc}	1.11^{b}	1.54^{bcde}
T_9: T_1 + BR foliar spray @ 0.1 ppm	0.40^{cd}	1.05^{e}	1.50^{ef}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	0.41^{b}	1.10^{bc}	1.54^{bc}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	0.40^{bcd}	1.05^{de}	1.53^{cde}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	0.41^{bc}	1.11^{b}	1.56^{b}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	0.41^{bc}	1.10^{bc}	1.53^{cde}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	0.46^{a}	1.20^{a}	1.63^{a}
SE(m)	0.004	0.013	0.012
CD (0.05)	0.013	0.039	0.035

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

4.3.2 Effect on Relative Growth Rate (RGR)

The data on effect of various growth regulators on RGR was recorded during 25-45 and 45-60 DAS and presented in Table 9. During the period of 25-45 DAS, the maximum growth rate recorded at treatment T_6 and T_{12} (0.10 mg g^{-1} d^{-1}) followed by similar growth rate recorded in treatments T_{10} and T_{12} (0.09 mg g^{-1} d^{-1}) which was on par with T_2 and T_{13} (0.87 mg g^{-1} d^{-1}).

RGR during 45-60 DAS was not significant. The highest RGR was recorded in treatment T_6 (0.102 mg g^{-1} d^{-1}) followed by T_5, T_8 and T_{14} (0.098 mg g^{-1} d^{-1}).

Table 9: Effect of various growth regulators on RGR at various growth stages of cowpea.

Treatments	Relative growth rate (RGR) (mg g^{-1} d^{-1})	
	25-45 DAS	45-60 DAS
T_1: Standard POP without TU (KAU, 2016)	0.080^{d}	0.080
T_2: Standard POP with TU foliar spray @ 500 ppm	0.087^{bc}	0.088
T_3: T_1 + SA foliar spray @ 50 ppm	0.080^{d}	0.070
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	0.080^{d}	0.095
T_5: T_1 + SA foliar spray @ 100 ppm	0.080^{d}	0.098
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	0.100^{a}	0.102
T_7: T_1 + SA foliar spray @ 150 ppm	0.080^{d}	0.095
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	0.080^{d}	0.098
T_9: T_1 + BR foliar spray @ 0.1 ppm	0.080^{cd}	0.090
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	0.090^{b}	0.096
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	0.083^{cd}	0.095
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	0.090^{b}	0.096
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	0.087^{bc}	0.097
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	0.100^{a}	0.098
SE(m)	0.002	0.008
CD (0.05)	0.005	NS

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

4.3.3 Effect on total dry matter production

The data on effect of various growth regulators on total dry matter production was recorded at 25, 45 and 60 DAS and presented in Table 10. At 25 DAS, maximum dry matter production recorded in T_{14} (1.97 g) followed by T_6 (1.89 g). T_6 was on par with T_8 (1.83 g) and T_{12} (1.85 g).

At 45 DAS, maximum total dry matter production was recorded in treatment T_{14} (15.51g) followed by T_6 (13.01g) which was the same pattern was followed at 25 DAS.

At 60 DAS, T_{14} (60.28 g) and T_6 (58.41 g) had maximum total dry matter production followed by T_{12} (44.20 g) which was on par with T_{10} (42.36 g). The least dry matter production recorded in control plant (28.95 g).

Table 10: Effect of various growth regulators on total dry matter production at various growth stages of cowpea.

Treatments	Mean total dry matter production(g)		
	25 DAS	**45 DAS**	**60 DAS**
T_1: Standard POP without TU (KAU, 2016)	1.52^{h}	8.17^{j}	28.95^{h}
T_2: Standard POP with TU foliar spray @ 500 ppm	1.65^{g}	9.04^{fg}	33.88^{g}
T_3: T_1 + SA foliar spray @ 50 ppm	1.64^{g}	8.22^{j}	34.19^{g}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	1.75^{de}	9.21^{ef}	40.39^{cd}
T_5: T_1 + SA foliar spray @ 100 ppm	1.69^{fg}	8.55^{hi}	39.13^{de}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	1.89^{b}	13.01^{b}	58.41^{a}
T_7: T_1 + SA foliar spray @ 150 ppm	1.66^{g}	8.43^{i}	35.84^{fg}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	1.83^{bc}	9.34^{e}	41.10^{cd}
T_9: T_1 + BR foliar spray @ 0.1 ppm	1.63^{g}	8.71^{h}	36.13^{fg}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	1.81^{cd}	10.02^{d}	42.36^{bc}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	1.66^{g}	8.94^{g}	37.90^{ef}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	1.85^{bc}	10.33^{c}	44.20^{b}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	1.73^{ef}	9.35^{e}	40.72^{cd}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	1.97^{a}	15.51^{a}	60.28^{a}
SE(m)	0.022	0.069	0.842
CD (0.05)	0.065	0.202	2.45

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

4.3.4 Effect on SPAD chlorophyll meter readings

The data on effect of various growth regulators on SPAD chlorophyll meter readings was recorded at 25, 45 and 60 DAS and presented in Table 11. At 25 DAS, highest reading was observed in treatments T_{14} (58.78) and T_6 (58.10) followed by T_{12} (56.70) which was on par with T_8 (56.37) and T_{10} (56.37).

At 45 DAS, maximum SCMR value recorded in T_{14} (64.73) and T_6 (64.12) followed by T_8 (59.03) which was on par with T_{12} (58.09). The lowest SCMR value noted in T_1 (50.8).

At 60 DAS, highest value was recorded in T_{14} (62.50) followed by T_6 (59.80). T_8 and T_{12} had same value (56.87) which was on par with T_4 (56.07) and T_{10} (55.87). The lowest SPAD reading was recorded in control (49.17).

Table 11: Effect of various growth regulators on SPAD chlorophyll meter readings at various growth stages of cowpea (SCMR).

Treatments	**Mean SCMR**		
	25 DAS	**45 DAS**	**60 DAS**
T_1: Standard POP without TU (KAU, 2016)	50.77^{g}	50.80^{h}	49.17^{h}
T_2: Standard POP with TU foliar spray @ 500 Ppm	52.83^{f}	54.40^{g}	52.70^{g}
T_3: T_1 + SA foliar spray @ 50 ppm	53.00^{f}	55.30^{fg}	54.33^{ef}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	54.67^{d}	56.77^{d}	56.07^{cd}
T_5: T_1 + SA foliar spray @ 100 ppm	54.13^{de}	56.60^{de}	55.03^{de}
T_6: Seed priming with 100 ppm SA +SA foliar spray @ 100 ppm	58.10^{a}	64.12^{a}	59.80^{b}
T_7: T_1 + SA foliar spray @ 150 ppm	53.82^{e}	55.60^{fg}	54.67^{de}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	56.37^{bc}	59.03^{b}	56.87^{c}
T_9: T_1 + BR foliar spray @ 0.1 ppm	52.83^{f}	54.53^{g}	52.87^{fg}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	56.37^{bc}	57.21^{cd}	55.87^{cde}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	54.46^{de}	55.13^{fg}	54.50^{de}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	56.70^{b}	58.09^{bc}	56.87^{c}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	55.73^{c}	56.67^{d}	54.90^{de}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	58.78^{a}	64.73^{a}	62.50^{a}
SE(m)	0.241	0.367	0.54
CD (0.05)	0.701	1.07	1.57

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

4.3.5 Effect on Relative water content (RWC)

The data on effect of various growth regulators on RWC was recorded at 25, 45 and 60 DAS and presented in Table 12. At 25 DAS, maximum RWC recorded in treatment T_6 (91.47 %) which was on par with T_{14} (89.32 %), T_8 (88.94 %), T_{12} (88.70 %) and T_5 (86.41 %). Minimum RWC experimented in T_2 (78.63%). At 45 DAS, there was no significant difference among the treatments. Although, T_6 (94.29 %) recorded maximum RWC followed by T_{14} (92.80 %). Minimum RWC recorded in control (82.99 %). At 60 DAS, the peak RWC was documented in T_6 (91.33 %) followed by T_{14} (89.00%). The lowest value of T_1 (83.00 %) distinguished from all other treatments.

Table 12: Effect growth regulators on RWC at various growth stages of cowpea.

Treatments	Mean relative water content (%)		
	25 DAS	45 DAS	60 DAS
T_1: Standard POP without TU (KAU, 2016)	82.83^{cde}	82.99	83.00^{bcd}
T_2: Standard POP with TU foliar spray @ 500 Ppm	78.63^{e}	83.08	78.33^{d}
T_3: T_1 + SA foliar spray @ 50 ppm	81.85^{de}	87.80	82.00^{cd}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	83.90^{bcde}	89.11	83.33^{bcd}
T_5: T_1 + SA foliar spray @ 100 ppm	86.41^{abcd}	91.94	86.33^{abc}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	91.47^{a}	94.29	91.33^{a}
T_7: T_1 + SA foliar spray @ 150 ppm	84.75^{bcde}	88.65	84.67^{bcd}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	88.94^{abc}	90.65	89.00^{ab}
T_9: T_1 + BR foliar spray @ 0.1 ppm	83.75^{bcde}	89.46	83.67^{bcd}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	84.01^{bcde}	90.92	84.00^{bcd}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	84.82^{bcde}	90.76	84.67^{bcd}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	88.70^{abc}	91.39	88.67^{ab}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	85.03^{bcde}	90.91	85.00^{abc}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	89.32^{ab}	92.80	89.00^{ab}
SE(m)	2.206	2.772	0.022
CD (0.05)	6.414	NS	0.064

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

4.3.6 Effect on Crop Growth Rate (CGR)

Table 13: Effect of various growth regulators on CGR at various growth stages of cowpea.

Treatments	Crop growth rate (g m^{-2} day^{-1})	
	25-45 DAS	**45-60 DAS**
T_1: Standard POP without TU (KAU, 2016)	7.39^{jk}	28.31^{g}
T_2: Standard POP with TU foliar spray @ 500 Ppm	8.21^{fg}	36.79^{f}
T_3: T_1 + SA foliar spray @ 50 ppm	7.32^{k}	38.46^{f}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	8.29^{efg}	46.18^{bcd}
T_5: T_1 + SA foliar spray @ 100 ppm	7.63^{ij}	45.48^{cd}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	12.36^{b}	67.25^{a}
T_7: T_1 + SA foliar spray @ 150 ppm	7.52^{jk}	40.42^{ef}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	9.35^{ef}	47.05^{bcd}
T_9: T_1 + BR foliar spray @ 0.1 ppm	7.87^{hi}	40.62^{ef}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	9.12^{d}	47.90^{bc}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	8.08^{gh}	42.91^{de}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	9.42^{c}	50.18^{b}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	8.47^{ef}	46.47^{bcd}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	15.05^{a}	66.31^{a}
SE(m)	0.083	1.47
CD (0.05)	0.242	4.26

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

The data on effect of various growth regulators on CGR was recorded during the period of 25-45 and 45-60 DAS and presented in Table 13. At 45 DAS, the highest CGR recorded in T_{14} (15.05 g m^{-2} day^{-1}) followed by T_6 (12.36 g m^{-2} day^{-1}). Minimum CGR was noted in T_5 (7.39 g m^{-2} day^{-1}).

At 60 DAS, maximum CGR was recorded in treatments T_6 (67.25 g m^{-2} day^{-1}) and T_{14} (66.31 g m^{-2} day^{-1}), followed by T_{12} (50.18 g m^{-2} day^{-1}). The lowest CGR was recorded in control (28.31 g m^{-2} day^{-1}).

4.3.7 Effect on Specific Leaf Weight (SLW)

The data on effect of various growth regulators on SLW was recorded at 25, 45 and 60 DAS and presented in Table 14. From the data it was clear that SLW had no significant variation among all the treatments.

Table 14: Effect of growth regulator on SLW (mg cm^{-2}) at various growth stages of cowpea.

Treatments	Specific Leaf Weight (mg cm^{-2})		
	25 DAS	45 DAS	60 DAS
T_1: Standard POP without TU (KAU, 2016)	3.67	7.00	7.33
T_2: Standard POP with TU foliar spray @ 500 ppm	4.33	7.67	7.67
T_3: T_1 + SA foliar spray @ 50 ppm	4.00	7.33	7.67
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	4.33	7.67	8.00
T_5: T_1 + SA foliar spray @ 100 ppm	4.00	7.67	7.33
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	5.00	8.33	8.00
T_7: T_1 + SA foliar spray @ 150 ppm	4.00	7.67	7.33
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	4.33	8.0	8.00
T_9: T_1 + BR foliar spray @ 0.1 ppm	4.00	7.67	8.00
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	4.33	7.67	8.00
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	4.00	7.67	8.00
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	4.67	8.00	8.33
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	4.00	7.67	8.00
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	4.67	8.00	8.33
SE(m)	0.28	0.42	0.28
CD (0.05)	NS	NS	NS

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

4.3.8 Effect on Fv/Fm, Y (II) and ETR

4.3.8.1 Ratio of variable fluorescence to maximum fluorescence (Fv/Fm)

Table 15: Effect of various growth regulator on Fv/Fm at various growth stages of cowpea.

Treatments	Fv/Fm		
	25 DAS	45 DAS	60 DAS
T_1: Standard POP without TU (KAU, 2016)	0.63^{e}	0.71	0.68
T_2: Standard POP with TU foliar spray @ 500 Ppm	0.72^{bc}	0.74	0.70
T_3: T_1 + SA foliar spray @ 50 ppm	0.72^{bc}	0.73	0.70
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	0.73^{b}	0.74	0.72
T_5: T_1 + SA foliar spray @ 100 ppm	0.72^{bc}	0.73	0.70
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	0.74^{ab}	0.75	0.73
T_7: T_1 + SA foliar spray @ 150 ppm	0.66^{d}	0.72	0.71
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	0.73^{b}	0.73	0.72
T_9: T_1 + BR foliar spray @ 0.1 ppm	0.70^{c}	0.73	0.69
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	0.73^{b}	0.74	0.72
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	0.70^{c}	0.74	0.71
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	0.74^{ab}	0.72	0.72
T_{13}: T_1+ BR foliar spray @ 0.5 ppm	0.73^{b}	0.73	0.71
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	0.76^{a}	0.75	0.73
SE (m)	0.008	0.012	0.012
CD (0.05)	0.024	NS	NS

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

The data on effect of various growth regulators on Fv/Fm was recorded at 25, 45 and 60 DAS and presented in Table 15. Direct measurement of chlorophyll fluorescence was recorded using portable stress kit. At 25 DAS, maximum value recorded in treatment T_{14} (0.76) which was on par with T_6 (0.74) and T_{12} (0.74). The least value recorded in control plants (0.63). At 45 and 60 DAS, there were no significant difference in Fv/Fm value among all treatments.

4.3.8.2 Effect on effective quantum yield Y(II)

Table 16: Effect of various growth regulators on effective quantum yield (Y (II)) at various growth stages of cowpea.

Treatments	YII		
	25 DAS	45 DAS	60 DAS
T_1: Standard POP without TU (KAU, 2016)	0.75^{e}	0.73^{e}	0.68^{e}
T_2: Standard POP with TU foliar spray @ 500 Ppm	0.77^{bcd}	0.74^{bcde}	0.70^{cde}
T_3: T_1 + SA foliar spray @ 50 ppm	0.76^{de}	0.73^{de}	0.70^{de}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	0.78^{bcd}	0.74^{bcde}	0.72^{abcd}
T_5: T_1 + SA foliar spray @ 100 ppm	0.76^{de}	0.74^{cde}	0.71^{bcd}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	0.80^{a}	0.76^{abc}	0.73^{ab}
T_7: T_1 + SA foliar spray @ 150 ppm	0.76^{cde}	0.74^{bcde}	0.70^{cde}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	0.78^{abc}	0.75^{abcd}	0.72^{abcd}
T_9: T_1 + BR foliar spray @ 0.1 ppm	0.76^{de}	0.73^{de}	0.71^{abcd}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	0.78^{ab}	0.76^{abc}	0.72^{abcd}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	0.77^{bcd}	0.74^{bcde}	0.71^{abcd}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	0.78^{abc}	0.76^{ab}	0.72^{abcd}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	0.77^{bcd}	0.74^{bcde}	0.73^{ab}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	0.80^{a}	0.77^{a}	0.73^{a}
SE(m)	0.007	0.007	0.009
CD (0.05)	0.021	0.021	0.027

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

The data on effect of various growth regulators on Y(II) was recorded at 25, 45 and 60 DAS and presented in Table 16. Y (II) value obtained at three growth periods were tabulated. At 25 DAS, the highest reading recorded in treatment T_{14} and T_6 with same reading (0.80) which were on par with T_8, T_{10} and T_{12} with same reading of (0.78). The lowest value recorded in T_1 (0.75).

At 45 DAS, T_{14} (0.77) recorded the highest Y(II) reading which was on par with T_6 and T_{12} with same reading (0.76).

At 60 DAS, the highest reading of observation noted on T_{14} (0.73). The least reading recorded in control plants (0.68).

4.3.8.3 Effect on Electron Transport Rate

Table 17: Effect of various growth regulators on ETR at various growth stages of cowpea.

Treatments	ETR		
	25 DAS	45 DAS	60 DAS
T_1: Standard POP without TU (KAU, 2016)	203.09^{e}	197.65^{f}	184.96^{d}
T_2: Standard POP with TU foliar spray @ 500 ppm	209.44^{bcd}	202.19^{bcdef}	193.12^{abc}
T_3: T_1 + SA foliar spray @ 50 ppm	205.81^{de}	199.47^{ef}	190.4^{cd}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	211.25^{bcd}	202.19^{bcdef}	196.75^{abc}
T_5: T_1 + SA foliar spray @ 100 ppm	205.81^{de}	201.28^{cdef}	192.21^{bc}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	217.60^{a}	207.63^{ab}	198.56^{ab}
T_7: T_1 + SA foliar spray @ 150 ppm	206.72^{cde}	200.37^{def}	191.31^{cd}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	212.16^{abc}	204.91^{abcdef}	196.75^{abc}
T_9: T_1 + BR foliar spray @ 0.1 ppm	205.81^{de}	200.37^{def}	191.31^{cd}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	212.16^{abc}	205.81^{abcd}	194.93^{abc}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	209.44^{bcd}	201.28^{cdef}	193.12^{abc}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	213.07^{ab}	206.72^{abc}	195.84^{abc}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	209.44^{bcd}	202.19^{bcdef}	194.03^{abc}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	217.60^{a}	209.44^{a}	199.45^{a}
SE(m)	1.93	1.97	2.41
CD (0.05)	5.62	5.73	6.99

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

The data on effect of various growth regulators on ETR was recorded at 25, 45 and 60 DAS and presented in Table 17. At 25 DAS, the superior value recorded in treatments T_{14} and T_6 (217.6) which was on par with T_8 (212.16), T_{10} (212.16) and T_{12}

(213.07). T_3, T_5 and T_9 were recorded same readings (205.81) and T_{11} and T_{13} had parallel recordings (209.44). The lowermost reading recorded in T_1 (203.09).

At 45 DAS, maximum value was recorded in T_{14} (209.44) which was on par with T_6 (207.63), T_{12} (206.72), T_{10} (205.81) and T_8 (204.91). The lowest value was recorded in control (197.65).

At 60 DAS, superior reading was noted in T_{14} (199.45) which was on par with T_6 (198.56), T_{12} (195.84), T_8 (196.75), T_4 (196.75), T_{10} (194.93), T_{13} (194.03), T_2 (193.12) and T_{11} (193.12). The lowest reading obtained in T_1 (184.96).

4.4 BIOCHEMICAL OBSERVATIONS

4.4.1 Effect on chlorophyll content

The data on effect of various growth regulators on chlorophyll content was recorded at 25, 45 and 60 DAS and presented in Table 18. At 25 DAS, maximum level of total chlorophyll content was recorded in treatments T_6 (2.03 mg g^{-1}) and T_{14} (1.99 mg g^{-1}) followed by T_8 (1.72 mg g^{-1}) and T_{12} (1.71 mg g^{-1}) which were on par with T_{11} (1.71 mg g^{-1}), T_{10} (1.70 mg g^{-1}), T_4 (1.67 mg g^{-1}), T_2 (1.66 mg g^{-1}), T_5 (1.63 mg g^{-1}) and T_9 (1.51 mg g^{-1}). The highest chlorophyll *a* content was recorded in treatments T_{14} (1.63 mg g^{-1}) and T_6 (1.62 mg g^{-1}) which was on par with T_{12} (1.53 mg g^{-1}), T_4 (1.47 mg g^{-1}) and T_8 (1.48 mg g^{-1}). Chlorophyll *b* was recorded the highest in treatment T_2 (0.5 mg g^{-1}) which was on par with T_6 (0.4 mg g^{-1}), T_{14} (0.35 mg g^{-1}) and T_4 (0.19 mg g^{-1}).

At 45 DAS, the highest total chlorophyll content was recorded in treatment T_6 (2.98 mg g^{-1}) which was on par with T_{14} (2.81 mg g^{-1}), T_8 (2.69 mg g^{-1}) and T_{12} (2.68 mg g^{-1}). Minimum level of total chlorophyll content was recorded in control (1.77 mg g^{-1}). Maximum chlorophyll *a* was recorded in T_6 (2.50 mg g^{-1}) which was on par with T_{14} (2.39 mg g^{-1}), T_{12} (2.37 mg g^{-1}), T_8 (2.35 mg g^{-1}) and T_5 (2.25 mg g^{-1}). There was no significant difference in chlorophyll *b* content.

At 60 DAS, the highest level of total chlorophyll content was recorded in treatment T_6 (1.92 mg g^{-1}) which was on par with T_{14} (1.84 mg g^{-1})). Maximum chlorophyll *a* was recorded in treatments T_6 (1.62 mg g^{-1}) and T_{14} (1.60 mg g^{-1}). Chlorophyll *b* was maximum recorded in T_2 (0.62 mg g^{-1}).

Table 18: Effect of various growth regulators on chlorophyll content at various growth stages of cowpea.

Treatments	Chlorophyll content (mg g^{-1})								
	25 DAS			45 DAS			60 DAS		
	Ch *a*	Ch *b*	Total	Ch *a*	Ch *b*	Total	Ch *a*	Ch *b*	Total
T_1: Standard POP without TU (KAU, 2016)	1.39^{bcd}	0.23^{bcde}	1.42^{d}	1.05^{h}	0.73	1.77^{h}	1.06^{e}	0.21^{cd}	1.26^{h}
T_2: Standard POP with TU foliar spray @ 500 ppm	1.15^{e}	0.50^{a}	1.66^{bc}	1.56^{fg}	0.72	2.27^{efg}	0.95^{f}	0.62^{a}	1.58^{fg}
T_3: T_1 + SA foliar spray @ 50 ppm	1.22^{de}	0.24^{bcde}	1.49^{cd}	1.90^{e}	0.36	2.32^{defg}	1.24^{d}	0.17^{d}	1.55^{g}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 Ppm	1.47^{abc}	0.19^{abc}	1.67^{bc}	2.19^{bcd}	0.34	2.59^{bcde}	1.40^{bc}	0.32^{bcd}	1.72^{cde}
T_5: T_1 + SA foliar spray @ 100 ppm	1.36^{bcd}	0.27^{bcde}	1.63^{bcd}	2.25^{abc}	0.56	2.61^{bcd}	1.41^{bc}	0.35^{b}	1.58^{fg}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 Ppm	1.62^{a}	0.40^{ab}	2.03^{a}	2.50^{a}	0.49	2.98^{a}	1.62^{a}	0.33^{b}	1.92^{a}
T_7: T_1 + SA foliar spray @ 150 ppm	1.35^{bcd}	0.27^{bcde}	1.60^{bcd}	1.93^{de}	0.39	2.45^{cdef}	1.38^{bc}	0.17^{d}	1.57^{g}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	1.48^{abc}	0.23^{bcde}	1.72^{b}	2.35^{ab}	0.40	2.69^{abc}	1.44^{b}	0.30^{bc}	1.77^{bc}
T_9: T_1 + BR foliar spray @ 0.1 ppm	1.34^{cd}	0.14^{e}	1.51^{bcd}	1.30^{gh}	0.39	2.07^{gh}	1.32^{cd}	0.32^{bc}	1.64^{efg}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 Ppm	1.36^{bcd}	0.18^{cde}	1.70^{bc}	2.02^{cde}	0.29	2.49^{cde}	1.40^{bc}	0.31^{bc}	1.71^{cde}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	1.35^{bcd}	0.16^{de}	1.71^{bc}	1.8^{ef}	0.39	2.17^{fg}	1.33^{cd}	0.32^{bc}	1.65^{defg}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 Ppm	1.53^{ab}	0.33^{bcd}	1.71^{b}	2.37^{ab}	0.45	2.68^{abc}	1.45^{b}	0.28^{bcd}	1.76^{bcd}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	1.40^{bcd}	0.31^{bcde}	1.49^{cd}	1.85^{e}	0.77	2.32^{defg}	1.37^{bc}	0.32^{bc}	1.69^{cdef}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	1.63^{a}	0.35^{abc}	1.99^{a}	2.39^{ab}	0.47	2.81^{ab}	1.60^{a}	0.31^{bc}	1.84^{ab}
SE(m)	0.060	0.610	0.076	0.100	0.130	0.107	0.040	0.040	0.039
CD (0.05)	0.190	0.180	0.220	0.280	NS	0.311	0.110	0.120	0.113

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

4.4.2. Effect on proline content

Table 19: Effect of various growth regulators on proline content at variousgrowth stages of cowpea.

Treatments	Proline content (µg g^{-1})		
	25 DAS	45 DAS	60 DAS
T_1: Standard POP without TU (KAU, 2016)	24.07^{a}	17.93^{a}	10.41^{a}
T_2: Standard POP with TU foliar spray @ 500 ppm	20.43^{d}	10.92^{bc}	7.99^{bc}
T_3: T_1 + SA foliar spray @ 50 ppm	22.57^{ab}	11.01^{bc}	8.56^{abc}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	20.49^{d}	9.32^{cd}	4.97^{fg}
T_5: T_1 + SA foliar spray @ 100 ppm	22.41^{abc}	10.07^{bcd}	6.93^{cde}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	14.25^{e}	7.16^{e}	4.17^{fg}
T_7: T_1 + SA foliar spray @ 150 ppm	22.14^{bcd}	10.87^{bc}	7.46^{cd}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	20.61^{cd}	9.29^{cd}	5.50^{ef}
T_9: T_1 + BR foliar spray @ 0.1 ppm	23.15^{ab}	11.83^{b}	9.61^{ab}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	21.94^{bcd}	9.92^{cd}	5.50^{ef}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	22.62^{ab}	10.92^{bc}	6.85^{cde}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	20.43^{d}	9.35^{cd}	4.17^{fg}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	22.55^{ab}	9.80^{cd}	6.04^{def}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	14.43^{e}	8.93^{de}	3.30^{g}
SE(m)	0.632	0.608	0.646
CD (0.05)	1.84	1.77	1.88

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

The data on effect of various growth regulators on proline content was recorded at 25, 45 and 40 DAS and presented in Table 19. The level of proline contentin plant sample analysed by fresh leaf weight basis. At 25 DAS, maximum proline content was recorded in control plants (24.07 µg g^{-1}) which was on par with T_9 (23.51 µg g^{-1}), T_{11} (22.62 µg g^{-1}), T_3 (22.57 µg g^{-1}), T_{13} (22.55 µg g^{-1}) and T_5 (22.41 µg g^{-1}).
T_6 (14.25 µg g^{-1}) and T_{14} (14.43 µg g^{-1}) contained least proline content.

At 45 DAS, the highest content of proline was recorded in control (17.93 µg g^{-1}) and lowest proline content was analysed in treatment T_6 (7.16 µg g^{-1}).

At 60 DAS, control plants showed more proline content (10.41 µg g^{-1}) which was on par with T_9 (9.61 µg g^{-}1) and T_3 (8.56 µg g^{-1}). Minimum proline content was recorded in T_{14} (3.3 µg g^{-1}).

4.5. YIELD AND POD QUALITY OBSERVATIONS

Table 20: Effect of various growth regulators on average number of pods per plant and average number of seeds per pod

Treatments	Pods per plant	Seeds per pod
T_1: Standard POP without TU (KAU, 2016)	16.20^{g}	15.17^{h}
T_2: Standard POP with TU foliar spray @ 500 ppm	17.80^{f}	15.63^{g}
T_3: T_1 + SA foliar spray @ 50 ppm	18.47^{f}	16.03^{f}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	20.20^{e}	16.53^{de}
T_5: T_1 + SA foliar spray @ 100 ppm	20.60^{de}	16.47^{de}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	25.93^{a}	17.27^{ab}
T_7: T_1 + SA foliar spray @ 150 ppm	18.73^{f}	16.40^{e}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	21.33^{cd}	16.63^{de}
T_9: T_1 + BR foliar spray @ 0.1 ppm	20.13^{e}	16.37^{ef}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	20.87^{cde}	16.80^{cd}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	20.20^{e}	16.37^{ef}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	22.33^{b}	17.03^{bc}
T_{13}: T_1+ BR foliar spray @ 0.5 ppm	21.73^{bc}	16.43^{e}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	25.60^{a}	17.57^{a}
SE(m)	0.328	0.122
CD (0.05)	0.954	0.354

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

4.5.1. Effect on average number of pods per plant

The average number of pods per plant recorded from representative plants (Table 20). The highest number of pods was obtained from treatments T_6 (25.93) and T_{14} (25.60) followed by T_{12} (22.33). T_{12} was on par with T_{13} (21.73). Minimum pod yield was recorded from control T_1 (16.20).

4.5.2. Effect on average number of seeds per pod

Effect of different treatments on average number of seeds per pod were recorded in Table 20. The highest number was recorded in treatment T_{14} (17.57) which was on par with T_6 (17.27). The least number of seeds per pod were obtained from T_1 (15.17).

Table 21: Effect of various growth regulators on average number of seeds per plant and weight of 100 seeds

Treatments	Average number of seeds perplant	Weight of 100 seeds (g)
T_1: Standard POP without TU (KAU, 2016)	255.07^{i}	13.07
T_2: Standard POP with TU foliar spray @ 500 Ppm	271.80^{i}	13.39
T_3: T_1 + SA foliar spray @ 50 ppm	295.13^{h}	13.15
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	336.27^{def}	13.60
T_5: T_1 + SA foliar spray @ 100 ppm	337.8^{def}	13.62
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	438.73^{a}	13.72
T_7: T_1 + SA foliar spray @ 150 ppm	306.73^{gh}	13.20
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	352.87^{cd}	13.68
T_9: T_1 + BR foliar spray @ 0.1 ppm	318.33^{fg}	13.26
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	347.33^{cde}	13.25
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	329.13^{efg}	13.37
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	377.07^{b}	13.60
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	361.88^{bc}	13.65
T_{14}: Seed priming with 0.3 ppm BL +BL foliar spray @ 0.5 ppm	438.40^{a}	13.88
SE(m)	7.937	0.345
CD	23.07	NS

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

4.5.3 Effect on average number of seeds per plant

Average number of seeds per plant recorded from representative plants. It showed variation among treatments(Table 21). T_6 (438.73) and T_{14} (438.40) recorded the highest seed number followed by T_{12} (377.07) and T_{13} (361.88).The least seed number per plant was recorded in control plants (255.07).

4.5.4 Effect on mean 100 seed weight

Treatment effect on weight of 100 seeds represented in Table 22. There was no significant difference among treatments. Maximum 100 seed weight recorded in treatment T_{14} (13.88 g) followed by T_6 (13.72 g). Minimum 100 seed weight recorded in control plants (13.07 g).

4.5.5. Effect on seed yield

Table 22: Effect of growth regulator on seed yield

Treatments	Seed yield		
	Per plant (g)	Per plot (kg)	Per ha (kg)
T_1: Standard POP without TU (KAU, 2016)	34.66^{g}	0.441^{i}	1759.85^{i}
T_2: Standard POP with TU foliar spray @ 500 ppm	38.34^{f}	0.500^{h}	2223.13^{h}
T_3: T_1 + SA foliar spray @ 50 ppm	38.80^{f}	0.499^{h}	2218.17^{h}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	43.04^{cd}	0.533^{d}	2355.44^{de}
T_5: T_1 + SA foliar spray @ 100 ppm	43.15^{cd}	0.528^{de}	2345.69^{de}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	56.22^{a}	0.567^{a}	2518.25^{a}
T_7: T_1 + SA foliar spray @ 150 ppm	40.45^{ef}	0.526^{ef}	2337.88^{e}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	43.63^{cd}	0.541^{c}	2403.37^{c}
T_9: T_1 + BR foliar spray @ 0.1 ppm	40.27^{ef}	0.507^{g}	2253.97^{g}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	44.41^{c}	0.532^{d}	2365.66^{d}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	41.36^{de}	0.521^{f}	2316.04^{f}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	47.14^{b}	0.547^{b}	2429.71^{b}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	44.60^{c}	0.530^{de}	2353.56^{de}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	55.28^{a}	0.563^{a}	2500.99^{a}
SE(m)	0.810	0.002	7.50
CD	2.35	0.005	21.81

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

The data on effect of various growth regulators on seed yield per plant/plot/ha was recorded and presented in Table 22. The highest mean seed yield per plant was recorded from treatments T_6 (56.22 g) and T_{14} (55.28 g) followed by T_{12} (47.14 g) and T_{13} (44.60 g). Maximum mean seed yield per plot was recorded from T_6 (0.567 kg) and T_{14} (0.563 kg) followed by T_{12} (0.547 kg). Among foliar spray of growth regulators T_{13} recorded the maximum seed yield (0.530 g). The least grain yield collected from T_1 (0.441 kg). The highest seed yield was recorded per hector from treatments T_6 (2518.25 kg) and T_{14} (2500.99 kg) followed by T_{12} (2429.71 kg). There was an increase in yield by 43.09 per cent in T_6 and 42.11 per cent in T_{14} compared with control plant, followed by T_{12} (38.09 per cent). With foliar spray of brassinolide
0.5 ppm yield increased by 33 percent and treatment with thiourea @ 500 ppm yield enhanced by 26.32 per cent over control plants.

4.6 ECONOMICS

4.6.1 Effect on net return and benefit cost ratio

The data on effect of various growth regulators on net returns and BCR was recorded and presented in Table 23. The highest net return was obtained in treatments T_6 (₹ 1.31 lakh ha^{-1}) followed by T_8 (₹ 1.22 lakh ha^{-1}). The lowest net returns are recorded in control (₹ 0.71 lakh ha^{-1}).

4.6.2 Benefit cost ratio

The benefit cost ratio on different treatments on the experimental study are presented in Table 23. The highest BCR recorded in treatment T_6 (2.86) followed by T_8 (2.73). The lowest BCR reported in treatment T_{11} (1.71).

Table 23: Effect of growth regulator on net returns and benefit cost ratio of cowpea

Treatments	Net returns (₹ Lakh ha^{-1})	Benefit cost ratio
T_1: Standard POP without TU (KAU, 2016)	0.71^{j}	2.00^{e}
T_2: Standard POP with TU foliar spray @ 500 ppm	1.07^{g}	2.52^{d}
T_3: T_1 + SA foliar spray @ 50 ppm	1.07^{d}	2.52^{d}
T_4: Seed priming with 100 ppm SA + SA foliar spray @ 50 ppm	1.18^{c}	2.67^{c}
T_5: T_1 + SA foliar spray @ 100 ppm	1.17^{c}	2.66^{c}
T_6: Seed priming with 100 ppm SA + SA foliar spray @ 100 ppm	1.31^{a}	2.86^{a}
T_7: T_1 + SA foliar spray @ 150 ppm	1.16^{c}	2.65^{c}
T_8: Seed priming with 100 ppm SA + SA foliar spray @ 150 ppm	1.22^{b}	2.73^{b}
T_9: T_1 + BR foliar spray @ 0.1 ppm	0.75^{i}	1.71^{j}
T_{10}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.1 ppm	0.84^{g}	1.80^{h}
T_{11}: T_1 + BR foliar spray @ 0.3 ppm	0.80^{h}	1.76^{i}
T_{12}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.3 ppm	0.89^{f}	1.84^{g}
T_{13}: T_1 + BR foliar spray @ 0.5 ppm	0.83^{g}	1.79^{h}
T_{14}: Seed priming with 0.3 ppm BR + BR foliar spray @ 0.5 ppm	0.95^{e}	1.90^{f}
SE(m)	0.007	0.008
CD	0.02	0.022

SA: Salicylic acid, BR: Brassinolide, TU: Thiourea

Discussion

5. DISCUSSION

Results obtained from the study on "Physiological effect of seed priming and foliar application of growth regulators in cowpea (*Vigna unguiculata* L.)" is tabulated in the preceding results chapter. The detailed analysis of the results are discussed with available review of literature in this chapter.

5.1 Effect on seed germination and early seedling growth

Seed germination and early seedling growth studies were carried out in petri plates and protrays. Minimum number of days taken for seed germination when the seeds primed with salicylic acid 100 ppm and brassinolide 0.1 ppm and 0.3 ppm which were reduced the time for germination by 16.75 per cent over control. It might be due to increase in the level of various enzymes activities like α- amylase, protease and lipase during seed sprouting which breakdown insoluble stored food material into soluble forms that would enhance seed germination and early emergence of seedlings. The result was in conformity with findings of Zhu *et al.* (2021) in a study with salicylic acid on rapeseed. 100 per cent seed sprouting and seed germination was observed in T_4 (salicylic 100 ppm) and T_7 (brassinolide 0.3 ppm) (Fig. 3). The similar results of T_4 using salicylic acid is also supported by Singh and Singh (2016) in tomato and Farooq *et al.* (2008) in maize. In T_7 brassinolide used for priming which issupported by findings of Vardhini and Rao (1998) in groundnut, Sairam (1994) in wheat and Semida and Rady (2014) in common bean. Minimum number of days takenfor 50 per cent germination was observed in T_7 (brassinolide 0.3 ppm) followed by T_4 (salicylic 100 ppm) and T_8 (brassinolide 0.5 ppm). Survival of all germinated seedlings was observed in T_4 when seedlings were primed with 100 ppm salicylic acidfollowed by T_7 (seeds priming in 0.3 ppm brassinolide), it might be due to treatment made seedlings less susceptible to soil borne pathogens, it was in harmony with study of El-Wakil *et al.* (2017) on salicylic acid pre-treatment study of potato seedling. Whole seedling dry weight was highest for seedlings primed with 0.3 ppm brassinolide followed by 0.1 ppm brassinolide. Brassinolide treatment increased cell division, cell elongation, vascular differentiation and enhances seedling growth which increased whole seedling dry weight. Which is supported by studies of Vardhini and

Rao, (1998) in groundnut. Increase in vigour index of seedlings by 48.88 per cent was recorded in plants treated with 0.3 ppm brassinolide over control. It could be due to increased shoot length by the action of cell division and cell expansion by brassinolide and thereby increasing whole dry weight of seedling which ultimately enhances seedling vigour index of treated seedlings (Fig. 4). This result is in accordance with Basit *et al.* (2021) where improved germination qualities and vigour index noted in rice seedlings under stress. Seedling vigour index increased by 28.5 percent when seeds primed in 100 ppm salicylic acid, this might be due to the function ofsalicylic acid in accelerating seed germination, as observed by Zhu *et al.* (2021) where 22.7 per cent increased vigour index was recorded in rapeseed with treatment of salicylic acid.

5.2. Effect on morphological and phenological parameters

Seed priming along with foliar treatments of various growth regulators at varying concentration influences morphological and phenological parameters of cowpea plants. Plant height, leaf area per plant, number of compound leaves per plant, number of days taken for 50 per cent flowering and first harvest, crop duration, pod characters, pest and disease incidences were altered with various treatments compared to control plants. Seed treatment with 0.3 ppm brassinolide followed by foliar spray of 0.5 ppm brassinolide showed better performance in various morphological characters. T_{14} was recorded increased plant height by 25.02, 23.69 and 13.81 per cent at 25, 45 and 60 DAS, respectively, over control (Fig. 5). It might be due to the response of brassinolide in enhancing cell expansion and cell elongation. Also, the function of brassinolide in inducing vascular differentiation causes greater plant height. The results were in conformity with experiments of Vardhini and Rao, (1998) in groundnut and Franck *et al.* (1998) in sweet pepper. Likewise, seed priming and foliarapplication of 100 ppm salicylic acid increased plant height by 21.35, 17.91 and 12.38per cent at 25, 45 and 60 DAS, respectively. The results might be due to function of salicylic acid in enhancing plant vegetative growth. The result is in conformity with the findings of Azoz and El Taher (2018) in cowpea where anatomical observation revealed the increase in size of stem diameter and leaf blade. Khandaker *et al.* (2011)

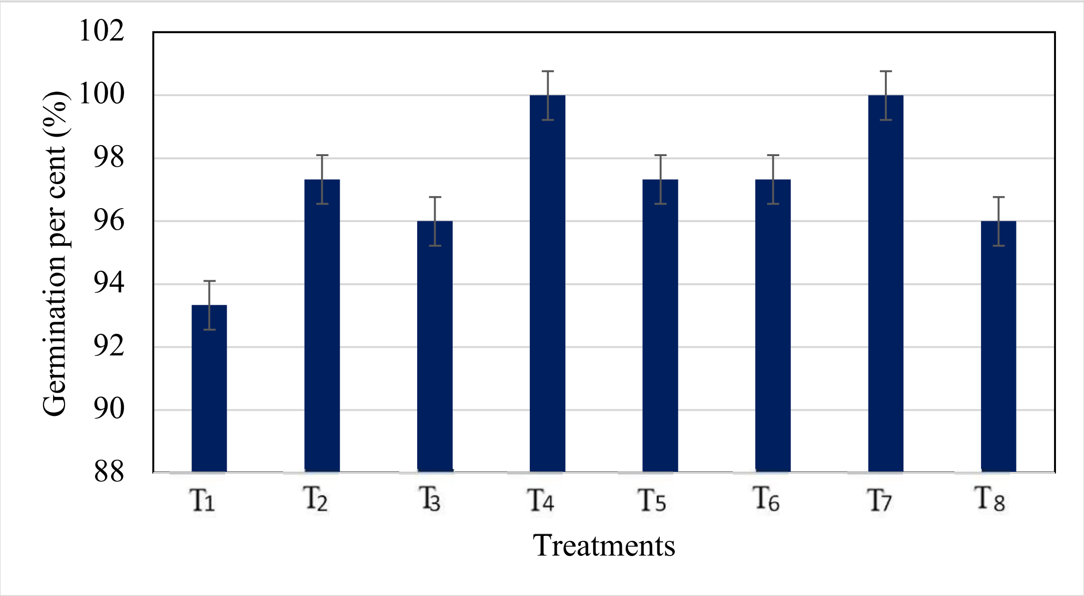


Figure 3: Effect of seed priming with various growth regulators on germination per cent of cowpea.

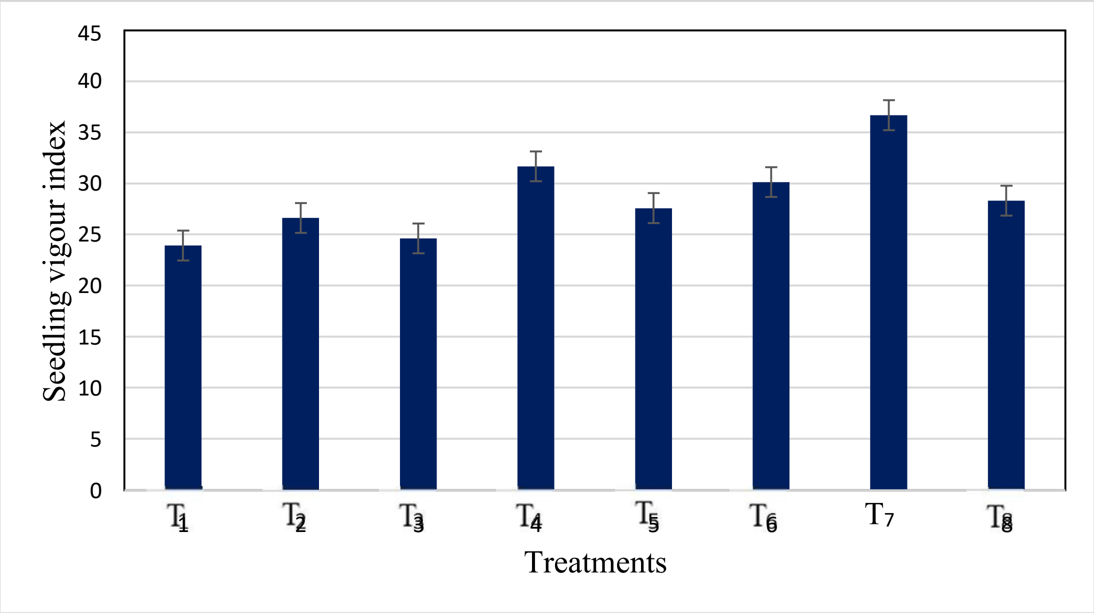


Figure 4: Effect of seed priming with various growth regulators on seedling vigour index of cowpea.

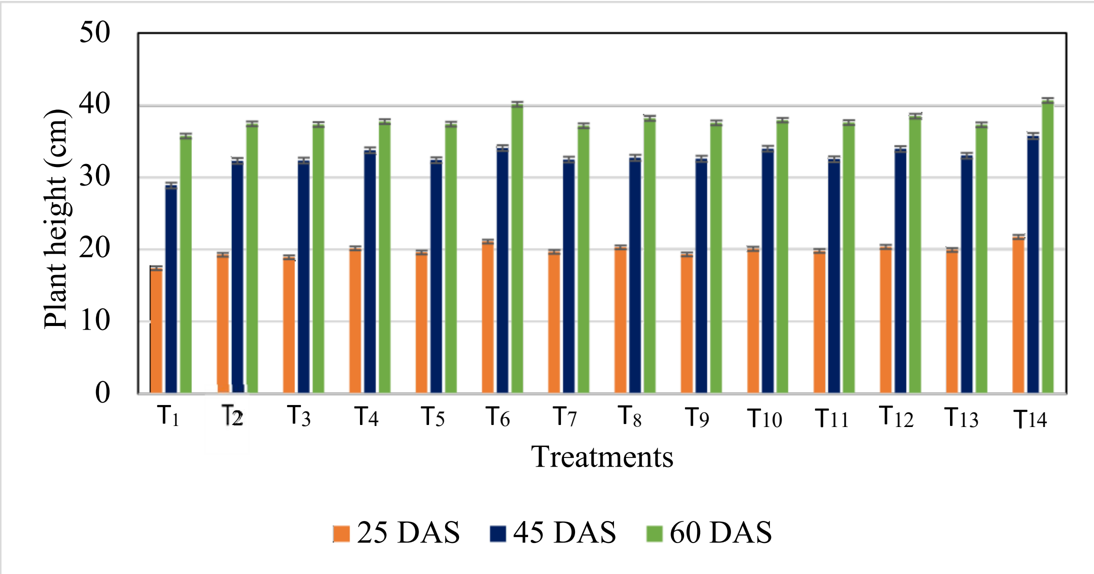


Figure 5: Effect of various growth regulators on mean plant height (cm) at different growth stages of cowpea.

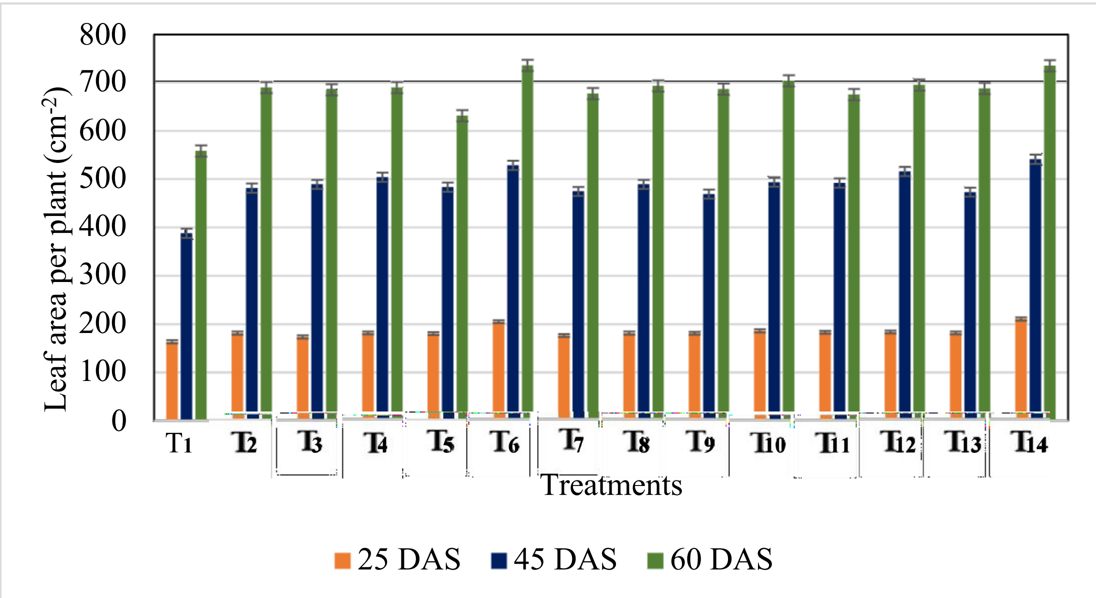


Figure 6: Effect of various growth regulators on mean leaf area per plant (cm^{-2}) at different growth stages of cowpea.

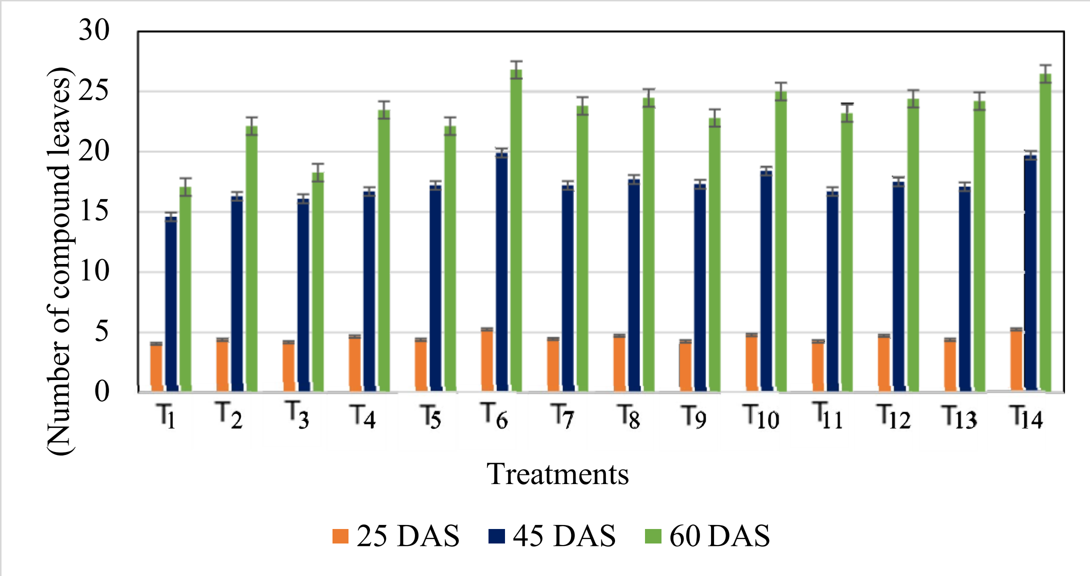


Figure 7: Effect of various growth regulators on mean number of compound leaves per plant at different growth stages of cowpea.

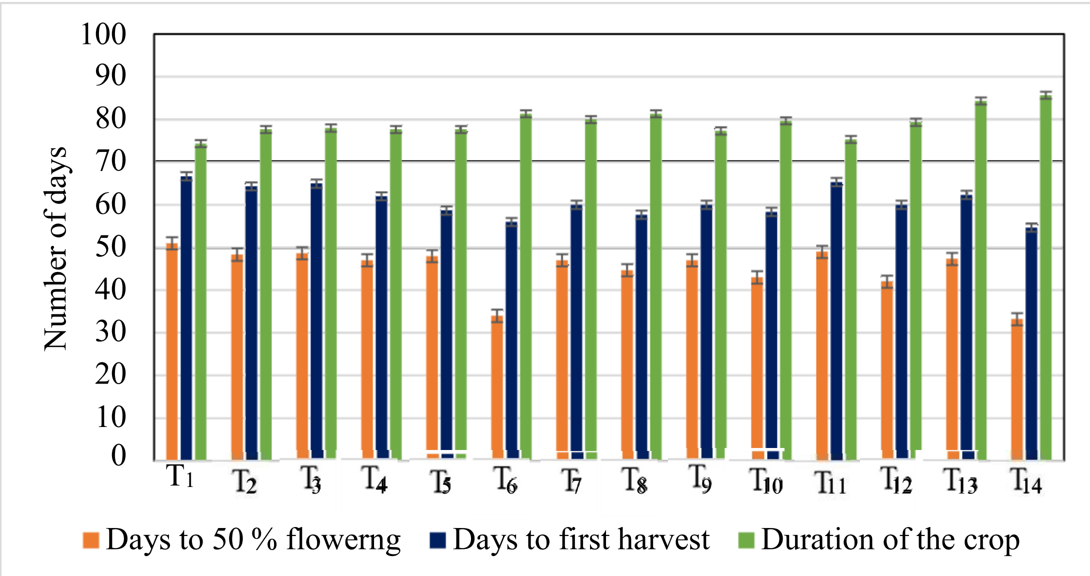


Figure 8: Effect of various growth regulators on days to 50% flowering, days to first harvest and duration crop.

in amaranthus, Kazemi (2014) in tomato and Gomaa *et al.* (2015) in lupine also confirmed the findings.

Highest leaf area per plant was recorded in combined application of seed priming and foliar spray over single foliar treatment only. Highest leaf area per plant was obtained from brassinolide treatment in T_{14} (seed priming with brassinolide 0.3 ppm followed by foliar spray at 0.5 ppm concentration) (Fig. 6). The increase in leaf area is associated with increased cell division and cell expansion. Brassinosteroid induced expansion of leaf area reported were by Asha and Lingakumar (2015) in cowpea and Sharma *et al*. (2016) in *Brassica juncea* L. Combined treatment of seed priming and foliar application of salicylic acid gave better results over foliar treatment only. Addition of seed priming along with foliar application of salicylic acid 100 ppm enhanced marginal leaf area by 13.48 per cent at 25 DAS, likewise in brassinolide 7.4 per cent marginal increase in leaf area was recorded. Which was confirmed with the conclusions of Gomaa *et al.,* (2015) in lupine plants.

Maximum number of compound leaves per plant was recorded when plants were subjected to treatment of salicylic acid with seed priming and foliar applicationat 100 ppm (Fig. 7). Here, 29.48 per cent increase in number of leaves per plant noticed at initial stage, followed by 36.5 per cent and 57 per cent increase towards its final growth period which is supported by study of Azoz and El Taher (2018) in cowpea, Khandaker *et al.* (2011) in amaranths, Mady (2009) in tomato, Gomaa *et al.* (2015) in lupine. Treatment with thiourea showed increase in leaves per plant by 8, 11.85 and 29.64 per cent over control at 25, 45 and 60 DAS respectively.

More number of days were taken for 50 per cent flowering in control plant. T_{14} and T_6 took fewer days for it. Minimum number of days taken for first harvest wasin T_6 (52.00 days) followed by T_{14} (53.67 days) (Fig. 8). It might be due to the role of salicylic acid in early flower induction and seed set. It confirmed by Khurana and Cleland, (1992) in study with *Lemna paucicostata*. Extended duration of crop was reported in plants subjected to treatments over control, which enhanced the productivecrop period. Among the treatments T_{14} had longer crop duration by 14.73 per cent more over control followed by T_6. The untreated population had nominal days of crop duration.

Regarding pod characters, its length and diameter were more on treatments of brassinolide and salicylic acid. These findings are supported by studies of Matwa *et al.* (2017) in green gram. Both T_{14} and T_6 had highest pod length and diameter. The findings point to the treatment effect of crops on various morpho-phenological parameters and these all altered the final yield.

Occurrence of pest and diseases were recorded less in treated plot over untreated plot. Stem fly (*Ophiomyia phaseoli*) attack was noticed during the initial seedling stage and pod borer (*Maruca vitrata*) incidence was noted during pod filling stage.

Similarly, foliar spray of thiourea exhibited better response over control. This result is in agreement the reports of Sahu *et al.* (1995), Premaradhya *et al.* (2018), Amin *et al.* (2014), Uddin *et al.* (2020) and Ravat and Makani (2015). Seed priming followed by foliar application of growth regulator like brassinolide, salicylic acid and thiourea at various growth stages influenced various morphological and phenological parameters of cowpea. These results pointing the growth stimulating properties of these chemical at varying concentrations. Collective effect of seed priming and foliar application of these growth regulators had better response over single method of treatment.

5.3. Effect of treatments on physiological observations

The study of effect of seed priming and foliar application of growth regulators altered various physiological parameters of cowpea. Maximum LAI was recorded in treatment T_{14} when brassinolide was given as priming at 0.3 ppm and foliar spray at 0.5 ppm (Fig. 9). It might be showing the trend due to increase in number of compound leaves with maximum leaf area by marginal leaf expansion withrespect to different treatments, which was supporting the findings of Sengupta *et al.* (2011) in green gram. Salicylic acid seed priming and leaf application at 100 ppm in treatment T_6 at three growth stages showed better LAI. This was in agreement with

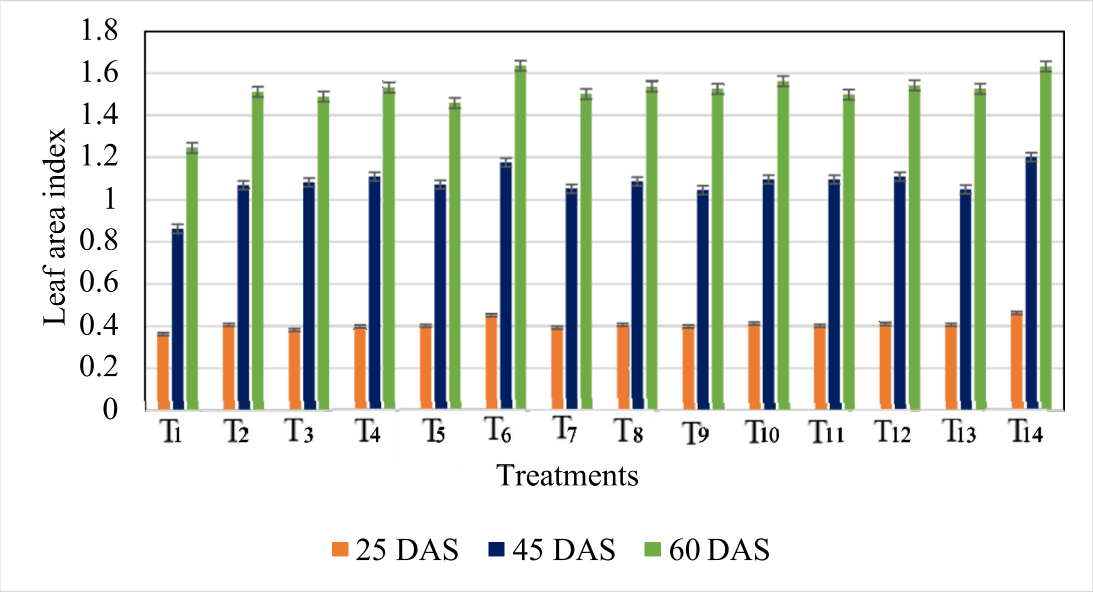


Figure 9: Effect of various growth regulators on mean leaf area index of plants at different growth stages of cowpea.

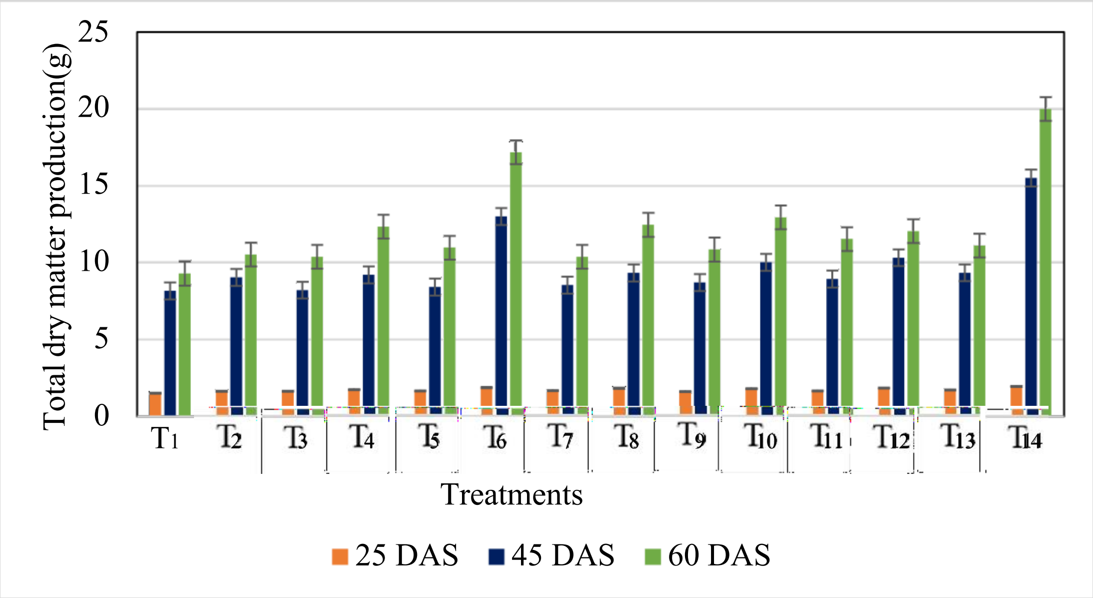


Figure 10: Effect of various growth regulators on mean total dry matter production (g) in plants at different growth stages of cowpea.

reports of Sadeghipour and Aghaei, (2012) in common bean and Tarighaleslami *et al.* (2012) in maize. Minimum LAI was recorded in T_1. With foliar application of thiourea LAI increased by 13.9, 24.42 and 20.8 per cent over control at 25, 45 and 60 DAS respectively.

Increase in RGR during initial vegetative phase was highest for T_{14} (brassinolide primed the seed at 0.3 ppm and foliar applied at 0.5 ppm) followed by T_6 (salicylic acid priming and foliar spray at 100 ppm) it could be due to increased leaf expansion and leaf area during initial stages which will helps to gather maximum solar radiation and it will enhance photosynthesis and net assimilate production and utilization for further growth. T_{10} and T_{12} followed the same result. During 25-45 DAS, highest RGR recorded in T_6 followed by T_{14}. At the end of reproductive stage the RGR reduced. The effect of salicylic acid on enhanced RGR might be due to its action on enhancing ion and nutrient uptake, exchange of gaseous through pores and boosted protein synthesis. These findings are in accordance with Hussein (2015) in okra. Here, the highest rate of growth was noted in T_6 (Salicylic acid 100 ppm seed priming and foliar spray) and least rate in control plants.

Total dry matter production varied with respect to treatment. Maximum dry matter production was obtained from T_{14} (0.3 ppm brassinolide seed treatment + 0.5 ppm foliar) followed by T_6 (salicylic acid 100 ppm seed priming + foliar) at different growth stages (Fig. 10). It might be due to increase in number of vegetative branches with a greater number of leaves and more leaf area, root length and nodulation, early initiation of reproductive branches etc. ultimately enhanced the total dry matter content of cowpea crops. Correspondingly, study of effect of brassinolide on total dry matter production reported by Coban and Baydar, (2016) in pepper mint, Sengupta *et al.* (2011) in green gram and the same effect of salicylic acid reported by Mady, (2009) in tomato, Fariduddin *et al.* (2003) in mustard and Kazemi (2014) in tomato. Likewise, T_8 and T_{12} also made more dry matter while least dry matter production was reported in T_1.

SPAD chlorophyll meter readings (SCMR) were highest for plants treated with both salicylic acid and brassinolide. T_{14} recoded 15.78 per cent more SCMR over control plants (Fig. 11). It seems to be due to involvement of brassinosteroids in molecular mechanisms of chlorophyll synthesis. These results are in conformity with Alyemeni and Al-Quwaiz, (2016) study in *Vigna radiata*. Maximum level of SCMR recorded in T_6 at 25, 45 and 60 DAS confirmed the treatment effect of salicylic acid. It might be due to the action of salicylic acid in increasing the level of various phenolics compounds and dyes. This was in conformity with the work done by Kazemi (2014) in tomato. T_8, T_{12}, T_4, T_5 and T_{11} also showed good response when growth regulators are applied. The lowest reading was recorded in control plants.

Relative water content (RWC) provide idea about health status of plant in terms of experiencing stress, here in study it was varied with treatment. Maximum RWC was recorded in T_6, which was on par with T_5, T_8, T_{12} and T_{14}. Brassinolide enhanced the RWC of plant compared to control plant which is supported by the reports of Yusuf *et al.* (2011) in boron stressed green gram, Edupuganti *et al.* (2019) in chickpea and salicylic acid treatment in stressed tomato (Rao *et al.*, 2012).

Crop growth rate of cowpea disclosed considerable increase in initial vegetative stage which was higher in T_{14} followed by T_6. During reproductive stage the pattern of CGR was same, highest in T_{14} and T_6, followed by T_8. Seed priming along with foliar application increased the CGR in plants. Increase in CGR might be due increase in leaf area, number of compound leaves, photosynthetic pigments etc. ultimately increased photosynthetic rate which leads to rise in the crop growth per unit area. The result is supported by findings of Tarighaleslami *et al.* (2012) in maize.

Specific leaf weight had no significant effect on treatments. Chlorophyll fluorescence Fv/Fm represents the ratio between variable fluorescence to its maximum fluorescence after dark adaptation, which indicate the efficiency of photosystem II. Treatment effect of growth regulator on Fv/Fm varied among treatments. At 25 and 45 DAS, T_6 and T_{14} recorded a trend of increase in chlorophyll fluorescence (5-6 per cent) over control, it might be due to increased efficiency of photosynthetic apparatus and chlorophyll pigments which ultimately enhanced the

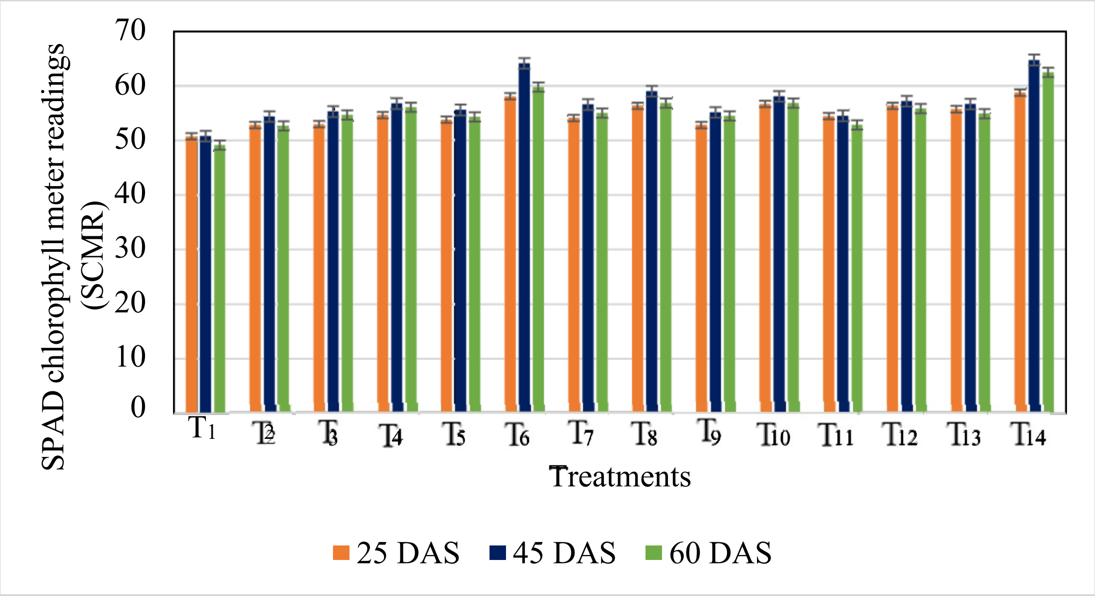


Figure 11: Effect of various growth regulators on mean SPAD chlorophyll meter readings of plants at different growth stages of cowpea.

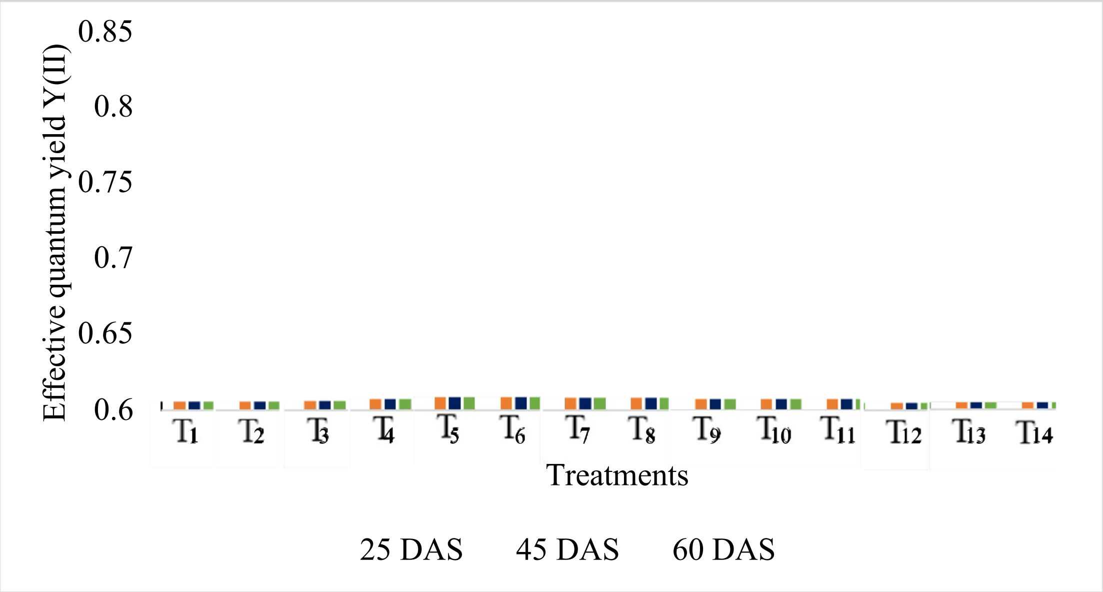


Figure 12: Effect of various growth regulators on mean effective quantum yieldY(II) of plants at different growth stages of cowpea.

Fv/Fm in treated plants. At the end of reproductive stage there was no treatment effect on Fv/Fm.

Photosynthetic quantum yield indicated the amount of energy used in photochemistry by photosystem II during a steady state photosynthetic light. Combination of seed priming and foliar spray of brassinolide reported the highest quantum yield in T_{14}, which was on par with T_6, T_8, T_{10} and T_{12} (Fig. 12). It could be due to positive response of treatment on rubisco activity which led to efficient photosynthesis and PS II quantum yield. The findings are supported by Yu *et al.* (2004) and Fariduddin *et al.* (2011) in cucumber plants.

Electron transport rate is associated with photosynthetic quantum yield, which is a measure of light adapted quantum yield. Seed priming with brassinolide at 0.3 ppm followed by foliar application at 0.5 ppm accelerated ETR in treated plants which was on par with seed priming and foliar feeding of salicylic acid at 100 ppm.

5.4. Effect of treatments on Biochemical observations

The analytical results of chlorophyll pigment in leaves at different stages revealed that treated plants displayed better result over control. Among the treatments,variation in chlorophyll *a*, chlorophyll *b* and total chlorophyll content were reported. Seed priming and leaf feeding of salicylic acid at 100 ppm in cowpea agreed the best result among, which was on par with brassinolide treatment by seed priming at 0.3 ppm and foliar application at 0.5 ppm. Highest chlorophyll *a* was recorded in treatment T_6 and T_{14} which was on par with T_{12} (1.53 mg g^{-1}), T_4 (1.47 mg g^{-1}) and T_8 (1.48 mg g^{-1}) (Fig. 13). Increase in the level of chlorophyll pigments in treated plants seems to be associated with rubisco activity, other phenolic compounds and dyes. Chlorophyll *b* was recorded the highest in treatment with thiourea at 500 ppm, which was on par with T_6, T_{14} and T_4. Treatment with thiourea increases over all photosynthetic rate in plant system, proper translocation of photosynthates and various metabolites ultimately enhances growth and final yield. These findings are in conformity with the results of Asha and Lingakumar (2015) in cowpea, Sairam (1994) and Sharma *et al*. (2016) in stressed *Brassica juncea* L plants.

Proline content of freshly harvested leaves of control plants recorded higher value compared to treated plants. Seed priming along with foliar application reduced the proline content. Proline content in plants reduced by 68.9 and 66.8 per cent in T_6 (salicylic acid 100 ppm priming + foliar) and T_{14} (brassinolide 0.3 ppm priming+ 0.5 ppm foliar) respectively over control during crop period, it might be due to the reduced stress in treated plants due to higher relative water content in plant cells. Which is supported by findings of Souri and Tohidloo, (2015) in salt stressed tomato, Coban and Baydar, (2016) in salt stressed peppermint. Niu *et al.* (2016) on temperature stressed *Leymus chinensis.*

5.5. Effect of treatments on yield and quality attributes

Treatments of various growth regulators influenced the final yield of cowpea. Seed priming followed by foliar application of growth regulators at specified concentration had considerable effect on yield attributes of cowpea. The average pod number per plant higher in cowpea, when seeds were primed and foliar sprayed with brassinolide and salicylic acid. Increased mean pod number per plant was recorded in T_6 by 60.06 per cent and in T_{14} by 58.02 followed by T_{12} 37.84 % per cent (Fig. 14). These findings confirmed by Sengupta *et al.* (2011) in green gram. Untreated plants recorded minimum number of pods per plant. Average number of seeds per pod where reported the same trend in outcome, T_{14} showed increased seed number by 15.82 per cent over control plants, which was on par with T_6 13.84 per cent. Seed numbers was less in control plants. Maximum number of average seeds per plant were from T_6 28.49 per cent and T_{14} 27.61 per cent followed by T_{10} 23.97 per cent, T_4 22.63 per cent compare to control. Weight of 100 seeds did not exhibit any appreciable significance in result, even though maximum seed weight recorded in T_{14} followed by T_6.

Final seed yield varied with treatments, the highest seed yield per plot obtained from T_6 (28.57%) and T_{14} (27.66 %), followed by T_{12} (24.04 %) over control plots. Seed yield per hector was reported maximum from T_6 (43 %) and T_{14} (42 %) followed by T_{12} (38 %) than control plants (Fig. 15). Seed priming and twice the foliar application of salicylic acid 100 ppm can be considered as one of the effective

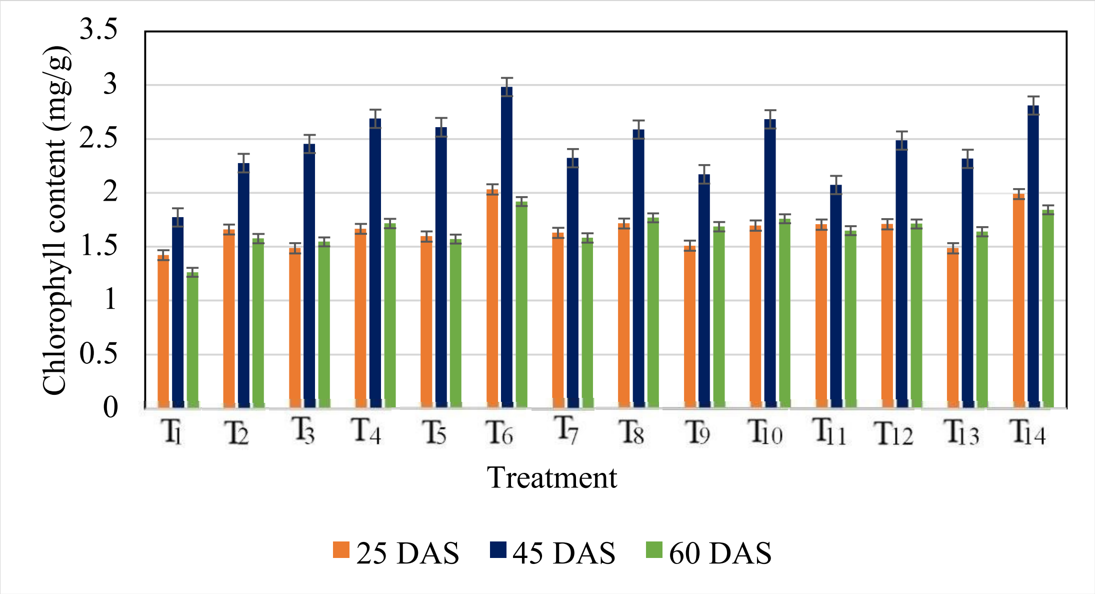


Figure 13: Effect of various growth regulators on mean chlorophyll content of plants at different growth stages of cowpea.

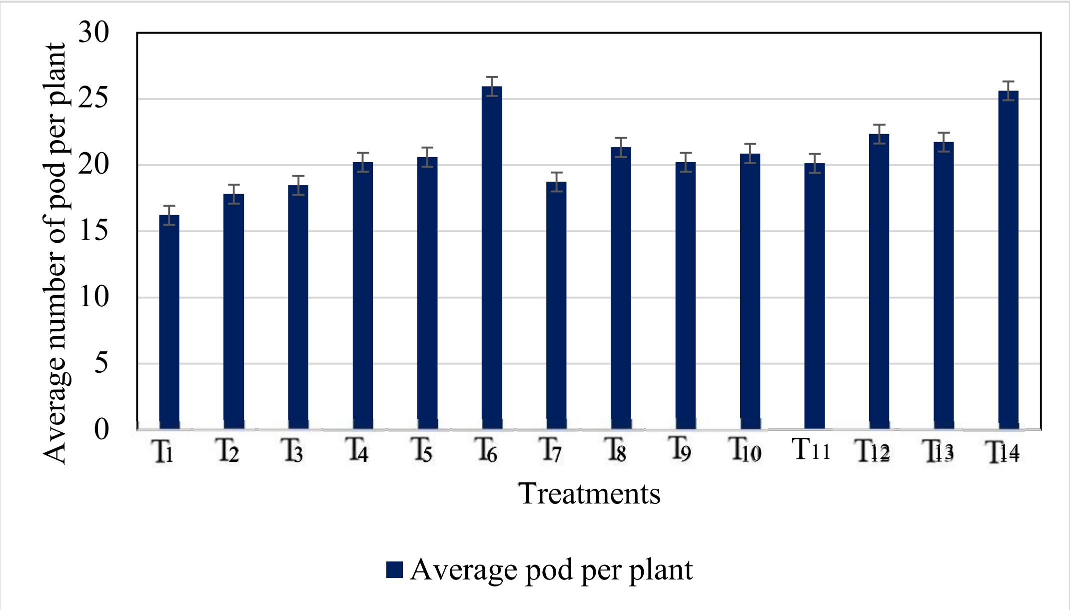


Figure 14: Effect of various growth regulators on mean number of pods per plants.

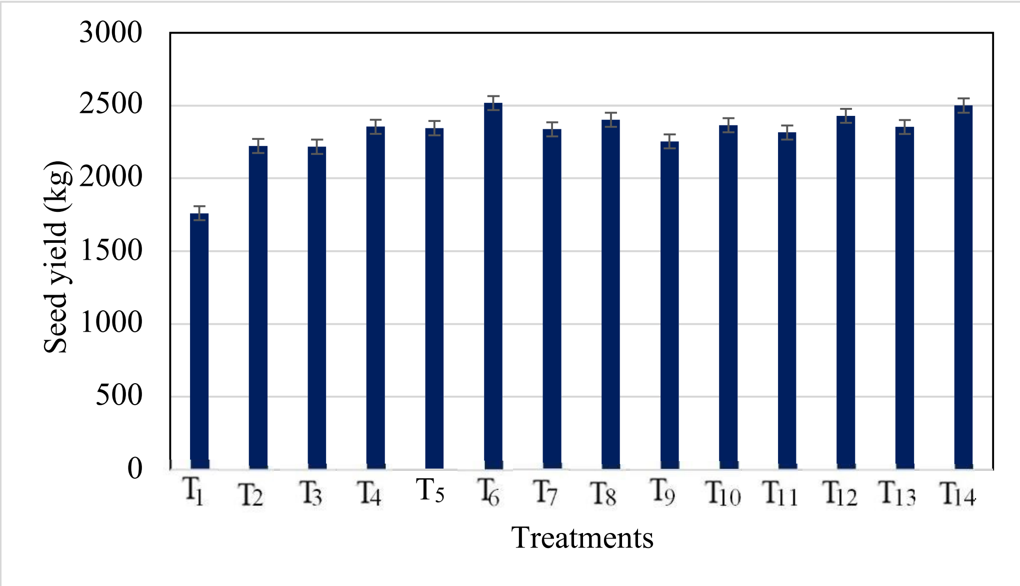


Figure 15: Effect of various growth regulators on mean seed yield of cowpea

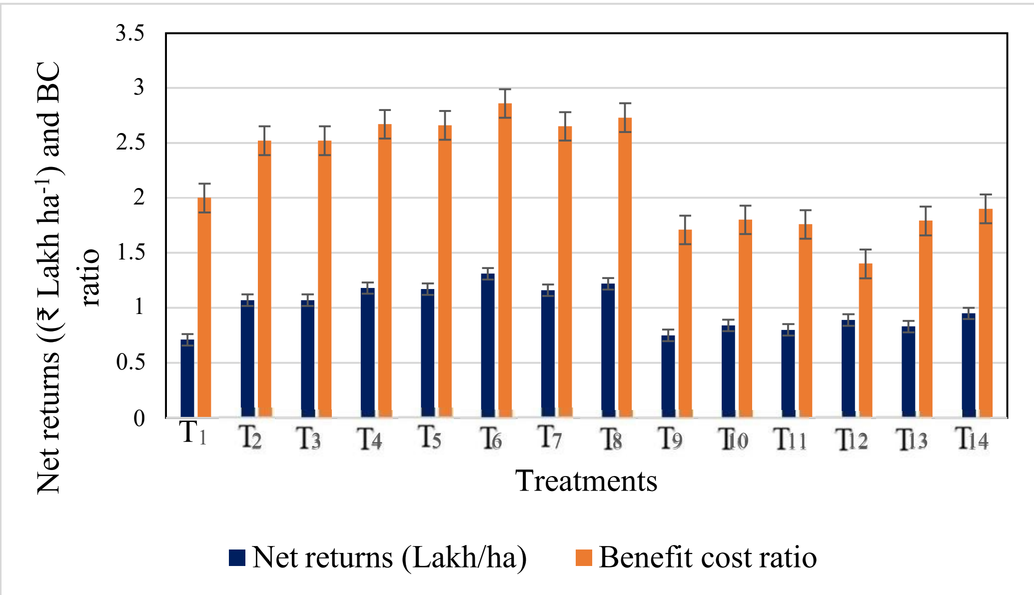


Figure 16: Effect of various growth regulators on mean net returns and benefit cost ratio

methods for enhancing yield in cowpea. Likewise, seed priming with 0.3 ppm brassinolide and twice the foliar application at 0.5 ppm concentration suitable for improving yield under field condition. Increased seed yield due to brassinolide application was reported by Vardhini and Rao (1998; 2001) in groundnut, Matwa *et al.* (2017) in green gram and Ritti *et al.* (2019) in bottle gourds. Similar findings in cowpea with salicylic acid was reported by Azoz and El Taher (2018), Khandaker *et al.* (2011) in red amaranths, Javaheri *et al.* (2012) in tomato.

5.6 Effect of treatments on economics

Seed priming followed by foliar application of growth regulators influenced initial plant growth to final yield. Different growth regulators having varying demand with respect to market value. Cost of cultivation and net returns vary with respect to inputs used. The highest net return reported in treatment T_6 followed by T_4. The minimal net returns obtained from control. Regarding BCR, the highest ratio obtained from T_6 followed by T_4. The least ratio recorded in T_{11} (Fig. 16).

Combined effect of seed priming and foliar application of salicylic acid at 100 ppm was enumerated as the best result for getting maximum net return and benefit: cost ratio. Among the foliar applications of salicylic acid, 100 ppm and 150 ppm performed well in terms of net returns. Seed priming and foliar application of brassinolide enhanced the overall net returns while due to highest price of the chemical the benefits: cost ratio was reduced.

The data and analysis pointing to the conclusion that seed priming along with foliar application of salicylic acid, brassinolide and thiourea significantly enhanced morpho-physiological, biochemical and yield attributes of cowpea crop. Among the treatments, seed priming along with foliar feeding of 100 ppm salicylic acid was bring into the best method for raising crops. As well, considering the net returns, brassinolide seed priming at 0.3 ppm and foliar application at 0.5 ppm was found to be the best method compare to other treatments.

Summary

6. SUMMARY

Sustainable agriculture imparts overall development of a country. Environment friendly tools and methods are to be followed to achieve this. Growth regulators are being used nowadays to achieve enhanced growth and development of plants and also to get high yield. Plant growth regulators are coming under chemical compounds which impart considerable effect on various morpho-phenological, physiological, biochemical and yield quality characteristics. These compounds are applied by external means in very small quantity and impart significant effect in crop system. Effective utilization of these chemical compounds at appropriate concentration can be used as a tool to enhance crop yield.

Here the present investigation entitled with "Physiological effect of seed priming and foliar application of growth regulators in cowpea (*Vigna unguiculata* L.)" was carried out under two experiments including lab study and field study at College of Agriculture, Padannakkad. Experiment 1 was on seed priming carried out in completely randomized design using cowpea seed variety PGCP-6, with 8 treatments *viz.,* hydropriming (T_1) as control, thiourea @ 500 ppm (T_2), salicylic acid @ 50 ppm (T_3), salicylic acid @ 100 ppm (T_4), salicylic acid @ 150 ppm (T_5), brassinolide @ 0.1 ppm (T_6), brassinolide @ 0.3 ppm (T_7) and brassinolide @ 0.5 ppm (T_8). The study carried out in three replications with 25 seeds in each treatment. Sprouts were transferred into protray filled with potting mixture and raised upto 10 days. Experiment 2 was carried out under field condition with 14 treatments and three replications studied in randomized block design. The best results obtained from Experiment 1 studied further under field conditions. The initial land preparation and soil test based nutrient management were carried out based on KAU package of practices, 2016. Overnight primed seeds were sown accordingly and foliar application of growth regulators was given at 20 and 40 DAS.

The results indicated that seed priming along with foliar application of growth regulators enhanced various morpho-physiological, biochemical and yield parameters of cowpea at different concentration. The salient finding of the study are:

- Minimum number of days required for seed germination was observed when seeds were primed in salicylic acid 100 ppm solution and brassinolide 0.1 ppm and 0.3 ppm. 100 per cent sprouting and germination was recorded when seeds primed with salicylic acid 100 ppm and brassinolide 0.3 ppm concentrations.
- Highest seedling vigour index was recorded when seeds were primed with brassinolide 0.3 ppm solution followed by salicylic acid 100 ppm which were on par with salicylic acid 150 ppm, brassinolide 0.1 ppm and 0.5 ppm.
- Seed priming and foliar application of brassinolide at 0.3 ppm and 0.5 ppm, respectively, imparted highest plant height at 25 DAS. At 45 and 60 DAS maximum plant height were recorded when combination of brassinolide seed priming at 0.3 ppm along with its foliar spray at 0.5 ppm and salicylic acid seed pre-treatment at 100 ppm followed by 100 ppm foliar.
- At 25, 45 and 60 DAS, maximum leaf area per plant were recorded in treatments of seed priming with 0.3 ppm brassinolide and foliar spray at 0.5 ppm. The results were on par with treatment of salicylic acid seed priming and foliar at 100 ppm. At 25 DAS, maximum number of compound leaves per plants was recorded when seed priming and foliar of brassinolide and salicylic acid respectively. Although, there were no significant difference at 45 DAS, maximum compound leaves were recorded when priming and foliar application of salicylic acid was at 100 ppm.
- Among treatments of foliar applications without considering seed priming, T_5 exhibit maximum increase in marginal leaf area by 10.37 per cent per plant.
- Days to 50 per cent flowering and days to first harvest can be minimised by seed priming and foliar feeding of growth regulators. The shortest number of days for 50 per cent flowering was observed in T_{14} treatment with brassinolide 0.3 ppm as seed priming and 0.5 ppm

foliar feeding, which were on par with seed priming and foliar application of salicylic acid at 100 ppm (T_{14}).

- Early harvest was recorded in treatment with salicylic acid 100 ppm seed priming and foliar, which were on par with brassinolide 0.3 ppm seed priming and 0.5 ppm foliar spray.
- Maximum crop duration was recorded when brassinolide was primed at 0.3 ppm and foliar given at 0.5 ppm (T_{14}) and salicylic acid 100 ppm seed priming and foliar (T_6), which was on par with brassinolide 0.5 ppm foliar spray alone. Use of growth regulators enhanced the durationof crop by reducing senescence which ultimately improved productive period of crop.
- Highest pod length was recorded in treatments of salicylic acid 100 ppm priming and foliar (T_6) and with brassinolide 0.3 ppm seed priming and 0.5 ppm foliar (T_{14}). The observations of salicylic acid priming at 100 ppm and foliar spray 150 ppm were on par with treatments of brassinolide 0.3 ppm pre-treated seed and its foliar spray 0.1 ppm and 0.3 ppm.
- Among the treatments, maximum pod diameter was recorded when salicylic acid priming and foliar application at 100 ppm concentration (T_6), which was on par with brassinolide treatment at 0.3 ppm seed priming and 0.5 ppm foliar application (T_{14}).
- Stemfly (*Ophiomyia phaseoli*) incidence reported in the initial seedling stages of crop and during pod filling stage pod borer (*Maruca vitrata*) attack was recorded and there was no any disease incidence.
- At 25, 45 and 60 DAS, maximum leaf area index was recorded when treatments given with seed priming in brassinolide at 0.3 ppm followed by foliar application at 0.5 ppm (T_{14}) and salicylic acid 100 ppm seed priming and foliar spray (T_6). It was in accordance with maximum leaf area per plant.
- At 25, 45 and 60 DAS, highest total dry matter production was recorded in treatment of brassinolide 0.3 ppm priming with 0.5 ppm foliar spray (T_{14}). This treatment followed by salicylic acid 100 ppm

pre-treatment and foliar application (T_6). Maximum vegetative growth together with plant height, more compound leaves, enhanced leaf area per plant, root growth resulted to maximum dry matter production in treated plants.

- Maximum relative growth rate was recorded at 25-45 DAS, in T_6 (salicylic acid at 100 ppm seed priming and foliar spray) and T_{14} (brassinolide seed primed at 0.3 ppm and 0.5 ppm foliar. Although, maximum RGR was recorded in salicylic acid 100 ppm seed priming and foliar spray (T_6) followed by brassinolide 0.3 ppm seed priming treatment and 0.5 ppm foliar spray (T_{14}). Increased vegetative growth including highest leaf area per plant, plant height, a greater number of compound leaves per plant gradually enhanced RGR. At reproductive stage, enhanced flowering and pod filling boosted the RGR.
- SPAD chlorophyll meter readings at 25 and 45 DAS were recorded, the highest in treatment with salicylic acid seed priming and foliar application @ 100 ppm (T_6) and brassinolide 0.3 ppm seed priming with 0.5 ppm foliar spray (T_{14}). At 60 DAS, SCMR recorded maximum in treatment of brassinolide 0.3 ppm seed priming with 0.5 ppm foliar (T_{14}). Synthesis of chlorophyll molecules and other pigments are accelerated by combination treatment seed priming and foliar spray of growth regulators.
- At 25 DAS, maximum relative water content was recorded in with salicylic acid 100 ppm seed priming and foliar application (T_6). This was on par with of treatments salicylic acid 100 ppm foliar spray (T_5), salicylic acid seed priming in 100 ppm with foliar spray of 150 ppm (T_8), brassinolide 0.3 ppm seed priming with 0.3 ppm (T_{12}) and 0.5 ppm foliar spray (T_{14}). At 45 DAS, although there was no significant effect on observations, maximum RWC was recorded on salicylic acid 100 ppm seed priming and foliar application (T_6). At 60 DAS, maximum RWC recorded in salicylic acid treatment @ 100 ppm seed priming and foliar spray. Which was on par with T_5, T_8, T_{12}, T_{13} and T_{14}. Here the growth regulators help to maintain water potential in

plant system by acting as a stress protectant, which finally maintained a healthy plant system.

- Maximum crop growth rate was recorded in plants treated with brassinolide 0.3 ppm seed priming and 0.5 ppm foliar spray (T_{14}). This was followed by observations of salicylic acid 100 ppm priming and foliar application (T_6). At 60 DAS, highest crop growth rate was recorded on salicylic acid @ 100 ppm seed priming and foliar spray (T_6) and brassinolide 0.3 ppm priming and 0.5 ppm foliar application (T_{14}). Per unit area increase in crop growth was higher in treatments due to enhanced shoot growth, root growth, leaves number, leaf area, reproductive parts etc.
- At 25 and 45 DAS, maximum Fv/Fm was recorded on salicylic acid seed priming and foliar application @ 100 ppm (T_6), which was on par with treatments of 100 ppm salicylic acid pre-treatment with its foliar spray @ 50 ppm (T_4), 150 ppm (T_8) solution and brassinolide 0.3 ppm priming with 0.1 ppm (T_{10}), 0.3 ppm (T_{12}) and 0.5 ppm (T_{14}) foliar sprays. Although, at 60 DAS, there was no significance among treatments, maximum Fv/Fm was recorded in salicylic acid @ 100 ppm seed priming followed by foliar spray (T_6) and brassinolide 0.3 ppm seed priming and 0.5 ppm foliar spray (T_{14}).
- Maximum Y(II) and ETR was recorded in treatment with brassinolide 0.3 ppm seed priming and 0.5 ppm foliar spray (T_{14}) and salicylic acid seed priming and foliar application @ 100 ppm (T_6).
- Maximum chlorophyll content was recorded when salicylic acid pre seed priming and foliar spray given at 100 ppm (T_6), which was on par with brassinolide seed priming at 0.3 ppm followed by foliar spray at 0.5 ppm (T_{14}). Treatments affect synthesis of chlorophyll molecules and other pigment systems which finally effect on seed yield.
- Maximum proline content was recorded in control plants. Least proline content was recorded in salicylic acid seed priming along with foliar application at 100 ppm (T_6) and brassinolide 0.3 ppm seed priming and foliar application at 0.5 ppm (T_{14}). Reduced level of proline content in

harvested leaves of treated plants revealed decreased stress in treated plants over control.

- ➢ Maximum number of pods per plant and number of seeds per pods were recorded in plants @ salicylic acid 100 ppm seed priming and foliar spray (T_6) and brassinolide 0.3 ppm seed priming and foliar application @ 0.5 ppm (T_{14}), which ultimately increased the seed yield per plant and per plot.

- ➢ Combination treatments of seed priming and foliar application had good impact on net returns. Treatments of seed priming with 100 ppm salicylic acid and twice the foliar spray of 100 ppm (T_6) followed by 150 ppm salicylic acid (T_8) recorded highest net returns. The least net return recorded in control. In brassinolide treatment, gross income was higher while because of greater expenditure net returns was reduced.

- ➢ With regard to BCR, highest BCR reported in salicylic acid 100 ppm seed priming and foliar (T_6) and the least ratio was from control. BCR for brassinolide treated plots were less due to higher cost of the brassinolide compare to other growth regulators.

FUTURE LINE OF WORK

- Optimizing concentration of salicylic acid and brassinosteroids for enhancing seedling vigour index of various crops.
- Identify appropriate concentration and combination of brassinosteroids and salicylic acid in various crops.
- Effective utilization of brassinosteroids and salicylic acid under stress condition.

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Appendix

Appendix I. Whether parameter during crop growing period of Experiment 2

Standard week	Temperature (^{0} C)		Rainfall (mm)
	Maximum	Minimum	
1 (Jan 1 - Jan 7)	32.71	21.93	6.00
2 (Jan 8 - Jan 14)	32.14	22.64	5.43
3 (Jan 15 - Jan 21)	31.86	22.50	0
4 (Jan 22 - Jan 28)	33.57	21.79	0
5 (Jan 29 – Feb 4)	32.86	20.90	0
6 (Feb 5 – Feb 11)	32.86	19.84	0
7 (Feb 12 – Feb 18)	32.86	21.69	0
8 (Feb 19 – Feb 25)	33.14	21.29	1.69
9 (Feb 26 – Mar 4)	33.14	23.71	0
10 (Mar 5 – Mar 11)	35.00	23.33	0
11 (Mar 12 – Mar 18)	34.07	24.57	0
12 (Mar 19 – Mar 25)	34.27	24.47	0.26
13 (Mar 26 – April 1)	34.34	25.71	0
14 (April 2 – April 8)	34.29	24.21	0
15 (April 9 – April 15)	34.64	25.57	0
16 (April 16 - April 22)	34.00	25.00	8.23
17 (April 23 - April 29)	34.21	26.01	0.03
18 (April 30 – May 6)	35.29	24.93	12.64
19 (May 7 – May 13)	33.81	25.00	6.21
20 (May 14 – May 20)	29.00	23.86	62.24
21 (May 21 – May 27)	31.57	24.14	4.96

PHYSIOLOGICAL EFFECT OF SEED PRIMING AND FOLIAR APPLICATION OF GROWTH REGULATORS IN COWPEA (*Vigna unguiculata* L.).

By

SWATHY P

(2019-11-207)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE
(PLANT PHYSIOLOGY)

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
PADANNAKKAD, KASARAGOD 671314
KERALA, INDIA

2022

ABSTRACT

Physiological effect of seed priming and foliar application of growth regulators in cowpea (*Vigna unguiculata* L.)

An investigation entitled "Physiological effect of seed priming and foliar application of growth regulators in cowpea (*Vigna unguiculata* L.)" was carried out in two experiments during the period 2020-2021. The objective of the study was elucidation of influence of seed priming and foliar application of growth regulators on morpho-physiological changes, yield and quality in cowpea (*Vigna unguiculata* L.).

Experiment-1 on seed germination and early seedling growth was conducted at College of Agriculture, Padannakkad. It was carried out in complete randomized design with eight treatments and three replications. Treatments included seeds primed with growth regulators *viz.,* thiourea @ 500 ppm (T_2); salicylic acid @ 50 ppm (T_3); salicylic acid @ 100 ppm (T_4); salicylic acid @ 150 ppm (T_5); brassinolide @ 0.1 ppm (T_6); brassinolide @ 0.3 ppm (T_7); brassinolide @ 0.5 ppm (T_8) and control (T_1) without growth regulators. Minimum number of days taken for 50 per cent seed germination in T_4, T_6 and T_7. 100 per cent seed sprouting and seed germination were recorded in T_4 and T_7. Maximum seedling survival was recorded in T_4 and T_7 which was on par with T_5 and T_6. Whole seedling dry weight and seedling vigour index were significantly enhanced in T_7 followed by T_4. Based on statistical analysis T_4 and T_7 were considered as best treatment for further field studies.

Experiment-2 was conducted as field study at Instructional Farm II, Karuvacheri, College of Agriculture, Padannakkad. The best seed priming results obtained from Experiment -1 were further studied under field condition in Experiment-2. It included 14 treatments *viz.,* Standard POP recommendation without thiourea (KAU, 2016) (T_1); standard POP with thiourea foliar spray @ 500 ppm (T_2); T_1 + salicylic acid foliar spray @ 50 ppm (T_3); seed priming with 100 ppm salicylic acid + salicylic acid foliar spray @ 50 ppm (T_4); T_1 + salicylic acid foliar spray @ 100 ppm (T_5); seed priming with 100 ppm

salicylic acid + salicylic acid foliar spray @ 100 ppm (T_6); T_1 + salicylic acid foliar spray @ 150 ppm (T_7); seed priming with 100 ppm salicylic acid + salicylic acid foliar spray @ 150 ppm (T_8); T_1 + brassinolide foliar spray @ 0.1 ppm (T_9); seed priming with 0.3 ppm brassinolide + brassinolide foliar spray@ 0.1 ppm (T_{10}); T_1 + brassinolide foliar spray @ 0.3 ppm (T_{11}); seed priming with 0.3 ppm brassinolide + brassinolide foliar spray @ 0.3 ppm (T_{12}); T_1 + brassinolide foliar spray @ 0.5 ppm (T_{13}) and seed priming with 0.3 ppm brassinolide + brassinolide foliar spray @ 0.5 ppm (T_{14}). The foliar sprays were given at 20 and 40 DAS.

Regarding morphological observations, T_6 and T_{14} significantly superior in plant height, leaf area per plant and number of compound leaves per plant at 25, 45 and 60 DAS. Minimum number of days taken for 50 per cent flowering in T_{14} which was on par with T_6. Minimum number of days for first harvest were recorded in T_6 and T_{14}. Duration of crop and greater number of productive flower setting were significantly superior in T_{14} and T_6 which was on par with T_{12}. Efficient translocation of assimilates increased pod length and pod diameter in T_6 which was on par with T_{14}.

Leaf area index and SPAD chlorophyll meter readings were maximum in T_6 and T_{14} at 25, 45 and 60 DAS. The increase in net photosynthetic area and assimilate translocation, enhanced the total dry matter production, relative growth rate and crop growth rate in T_{14} followed by T_6 during vegetative stage and reproductive stage as well. Maximum relative leaf water content was observed in T_6 which was on par with T_5, T_8, T_{12} and T_{14} at 25 DAS and T_5, T_8, T_{12}, T_{13} and T_{14} at 60 DAS. The ratio of variable fluorescence to maximum fluorescence (Fv/Fm) was recorded maximum in T_{14} which was on par with T_6 and T_{12} at 25 DAS. The maximum effective quantum yield of PS II (Y (II)) were recorded maximum in T_6 and T_{14} which were on par with T_{10} and T_{12} at 25 DAS. At 45 DAS, T_{14} was on par with T_6, T_8, T_{10} andT_{12}. At 60 DAS, T_{14} was on par with T_4, T_6, T_8, T_9, T_{10}, T_{11}, T_{12} and T_{13}. Electron transport rate (ETR) were observed maximum in T_6 and T_{14} which were on par with T_8, T_{10} and T_{12} at 25 DAS. At 45 DAS, T_{14} was on par with T_6, T_8, T_{10} and T_{12}. At 60 DAS, T_{14} was on par with T_2, T_4, T_6, T_8, T_{10}, T_{11}, T_{12}, and T_{13}.

A perusal of biochemical data showed that T_6 and T_{14} had maximum leaf chlorophyll content at 25 DAS. At 45 DAS, T_6 had the highest chlorophyll content which was on par with T_8, T_{12} and T_{14}. At 60 DAS, maximum chlorophyll content was recorded in T_6 which was on par with T_{14}. Maximum proline content was recorded in T_1 at 25, 45 and 60 DAS.

Regarding yield parameters average number of pods per plant and average number of seeds per plant were recorded maximum in T_6 and T_{14}, Seed yield was found to be increased by 43 per cent in T_6 when salicylic acid @ 100 ppm seed priming and foliar application were given and 42.11 per cent increase in seed yield was noticed in T_{14} when seed priming was given with brassinolide @ 0.3 ppm and foliar spray at 0.5 ppm. Among only foliar spray of growth regulators without considering seed priming, brassinolide @ 0.5 ppm (33.74 per cent) and salicylic acid @ 100 ppm (33.29 per cent) were recorded maximum increase in seed yield. Foliar spray of thiourea was increased the yield by 26.32 per cent over control.

Maximum net returns were obtained from treatment of seed priming and foliar spray with salicylic acid 100 ppm (T_6) followed by T_8 and T_5. Even though, the gross returns were maximum from brassinolide treated plants, the net return was less due to higher cost of brassinolide. The highest BC ratio was from salicylic acid treated plants T_6 (2.86) followed by T_8 (2.73) and T_4 (2.67). Among foliar sprayed treatments T_5 recorded maximum BC ratio (2.66) and thiourea treatment recorded BC ratio 2.52. Results of the study revealed that seed priming and foliar spray of salicylic acid 100 ppm was significantly superior to all other treatments with respect to yield and net return.

It can be concluded that combination of seed priming and foliar application of salicylic acid and brassinolide enhanced various morpho-phenological, physiological, biochemical and yield parameters of cowpea. Among the treatments seed priming and foliar application of salicylic acid 100 ppm and seed priming with brassinolide @ 0.3 ppm and 0.5 ppm foliar spray showed the best results. Among foliar spray without seed priming salicylic acid 100 ppm and brassinolide 0.5 ppm superior in all parameters. Maximum economic returns were recorded from salicylic acid treated plants, even though highest seed yield and gross returns observed from brassinolide treatment because of high cost of chemical, net returns were minimised. Therefore, seed priming and foliar application of salicylic acid (100 ppm) significantly enhanced seed yield and net returns in cowpea.

സംക്ഷിപ്തം

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വളർച്ചാ ത്വരിത പദാർത്ഥങ്ങൾ ഉപയോഗിച്ചുള്ള പ്രാഥമിക വിത്തു പരിചരണവും തുടർന്നുള്ള ഇലകളിലെ തളിക്കലും പയറുചെടികളിൽ ഉണ്ടാക്കുന്ന ശാരീരിക മാറ്റങ്ങൾ എന്ന തലക്കെട്ടിൽ ഒരു പരീക്ഷണം 2020-2021 കാലയളവിൽ സംഘടിപ്പിച്ചു. പരീക്ഷണത്തിന്റെ ഉദ്ദേശ്യം വിത്തു നടുന്നതിനു മുൻപ് വളർച്ചാ ത്വരിത പദാർത്ഥങ്ങൾ ഉപയോഗിച്ചുള്ള വിത്ത് പരിചരണവും അവ ഇലകളിൽ തളിക്കുന്നത് വഴി പയറുചെടികളിൽ ഉണ്ടാകുന്ന രൂപവിജ്ഞാനീയവും ശരീരശാസ്ത്രപരവുമായ മാറ്റങ്ങൾ മനസിലാക്കലും അതുവഴി ഉണ്ടാകുന്ന വിളവും വിളഗുണനിലവാരവും അപഗ്രഥിക്കലുമാണ്.

പരീക്ഷണം ഒന്ന് വിത്ത് മുളപ്പിക്കലും ബീജാങ്കുരത്തിന്റെ വളർച്ചാ മാറ്റങ്ങളും അപഗ്രഥിക്കലുമാണ്. ഇത് പടന്നക്കാട് കാർഷിക കോളേജിൽ വെച്ച് പൂർത്തിയാക്കി. പൂർണമായും ആകസ്മികമായ പദ്ധതിയിൽ എട്ട് പരിചരണ മുറകളായി മൂന്നു ആവർത്തനങ്ങളിലായി പൂർത്തീകരിച്ചു. പഠനത്തിനായി അവലംബിച്ച പരിചരണ മുറകളുടെ വിവരണം: പ്രാഥമിക വിത്തു പരിചരണമായി വിത്ത് രാത്രി സമയത്ത് ലായനികളിൽ മുക്കി വെക്കൽ, തയോയൂറിയ @ 500 ppm (T_2), സാലിസിലിക് അമ്ലം @ 50 ppm (T_3), സാലിസിലിക് അമ്ലം @ 100 ppm (T_4), സാലിസിലിക് അമ്ലം @ 150 ppm (T_5), ബ്രാസ്സിനൊലിടെ @ 0.1 ppm (T_6), ബ്രാസ്സിനൊലിടെ @ 0.3 ppm (T_7), ബ്രാസ്സിനൊലിടെ @ 0.5 ppm (T_8), വെള്ളത്തിൽ (T_1).

അൻപത് ശതമാനം വിത്ത് മുളക്കുന്നതിന് കുറഞ്ഞ ദിവസങ്ങൾ വേണ്ടിവന്നുള്ളൂ T_4, T_6, T_7 എന്നിവയിൽ. ബീജാങ്കുരത്തിന്റെ കരുത്തു സൂചിക T_4 ലും T_7 ലും മുന്നിട്ട് നിന്നു. T_4 ലും T_7 ലും നൂറു ശതമാനവും വിത്ത് കിളിർത്തു വന്നതായി കാണപ്പെട്ടു. മുളച്ച തൈച്ചെടികളുടെ മെച്ചപ്പെട്ട അതിജീവനം T_4, T_7 എന്നിവ രേഖപ്പെടുത്തി. മൊത്തം ഉണങ്ങിയ ബീജാങ്കുരത്തിന്റെ ഭാരവും ബീജാങ്കുരത്തിന്റെ കരുത്തും T_7 ൽ സാരമായി വർധിച്ചതായി കാണപ്പെട്ടു. T_7 ന് ശേഷം T_4 ൽ ഇവ കൂടുതലായി കാണപ്പെട്ടു. സ്ഥിരവിവരശാസ്ത്രത്തിന്റെ

അടിസ്ഥാനത്തിൽ T_4 ഉം T_7 ഉം മുന്തിയ വിത്ത് പരിചരണ മുറകളായി പരിഗണിച്ച് വിളഭൂമിയിൽ പരീക്ഷണത്തിനായി അവലംബിച്ചു.

പരീക്ഷണം രണ്ട് ആസൂത്രണം ചെയ്യുന്നതിന് വേണ്ടി പടന്നക്കാട് കാർഷിക കോളേജിലെ കരുവാച്ചേരി ശിക്ഷണ കൃഷിഭൂമി രണ്ട് തെരഞ്ഞെടുത്തു. പരീക്ഷണം ഒന്നിൽ നിന്നും ലഭിച്ച മികച്ച ഫലം പരീക്ഷണം രണ്ടിൽ പതിനാല് പരിചരണമുറയായി അവലംബിച്ചു. അവയുടെ വിവരങ്ങൾ: കേരള കാർഷിക സർവകലാശാല ശുപാർശ പ്രകാരം തയോയൂറിയ കൂടാതെ (T_1), കേരള കാർഷിക സർവകലാശാല ശുപാർശയുടെ കൂടെ തയോയൂറിയ @ 500 ppm (T_2), T_1 + സാലിസിലിക് അമ്ലം ഇലകളിൽ തളിക്കൽ @ 50 ppm (T_3), 100 ppm സാലിസിലിക് അമ്ലം കൊണ്ടുള്ള പ്രാഥമിക വിത്ത് പരിചരണം + സാലിസിലിക് അമ്ലം ഇലകളിൽ തളിക്കൽ @ 50 ppm (T_4), T_1 + സാലിസിലിക് അമ്ലം ഇലകളിൽ തളിക്കൽ @ 100 ppm (T_5), 100 ppm സാലിസിലിക് അമ്ലം കൊണ്ടുള്ള പ്രാഥമിക വിത്ത് പരിചരണം + സാലിസിലിക് അമ്ലം ഇലകളിൽ തളിക്കൽ @ 100 ppm (T_6), T_1 + സാലിസിലിക് അമ്ലം ഇലകളിൽ തളിക്കൽ @ 150 ppm (T_7), 100 ppm സാലിസിലിക് അമ്ലം കൊണ്ടുള്ള പ്രാഥമിക വിത്ത് പരിചരണം + സാലിസിലിക് അമ്ലം ഇലകളിൽ തളിക്കൽ @ 150 ppm (T_8), T_1 + ബ്രാസ്സിനൊലിടെ ഇലകളിൽ തളിക്കൽ @ 0.1 ppm (T_9), 0.3 ppm ബ്രാസ്സിനൊലിടെ കൊണ്ടുള്ള പ്രാഥമിക വിത്ത് പരിചരണം + ബ്രാസ്സിനൊലിടെ ഇലകളിൽ തളിക്കൽ @ 0.1 ppm (T_{10}), T_1 + ബ്രാസ്സിനൊലിടെ ഇലകളിൽ തളിക്കൽ @ 0.3 ppm (T_{11}), 0.3 ppm ബ്രാസ്സിനൊലിടെ കൊണ്ടുള്ള പ്രാഥമിക വിത്ത് പരിചരണം + ബ്രാസ്സിനൊലിടെ ഇലകളിൽ തളിക്കൽ @ 0.3 ppm (T_{12}), T_1 + ബ്രാസ്സിനൊലിടെ ഇലകളിൽ തളിക്കൽ @ 0.5 ppm (T_{13}), 0.3 ppm ബ്രാസ്സിനൊലിടെ കൊണ്ടുള്ള പ്രാഥമിക വിത്ത് പരിചരണം + ബ്രാസ്സിനൊലിടെ ഇലകളിൽ തളിക്കൽ @ 0.5 ppm (T_{14}). വിത്ത് നട്ടത്തിന് ശേഷം 20, 40 ദിവസങ്ങളിലായി വളർച്ചാ ത്വരിത പദാർത്ഥങ്ങളുടെ ലായനികൾ ഇലകളിൽ തളിച്ച് കൊടുത്തു.

വിത്ത് നട്ട് 25, 45, 60 ദിവസങ്ങളിൽ രൂപവിജ്ഞാനീയ അവലോകനത്തിൽ T_6 ഉം T_{14} ഉം മുന്തിയ ചെടി ഉയരവും ഇലകളുടെ വിസ്തൃതിയും ഇലകളുടെ എണ്ണവും രേഖപ്പെടുത്തി. ചെടികളിൽ അൻപത് ശതമാനം പൂക്കൾ വിരിയുന്നതിന് T_{14} ഏറ്റവും കുറഞ്ഞ ദിവസങ്ങൾ എടുത്തു. ഇത് T_6 ന് തുല്യമായിരുന്നു. കുറഞ്ഞ ദിവസങ്ങൾകൊണ്ട് T_6 ലും T_{14} ലും ആദ്യവിളവെടുപ്പ് രേഖപ്പെടുത്തി. വിളകളുടെ കൂടിയ കാലദൈർഘ്യവും കൂടുതൽ പുഷ്പിക്കലും കായ്കളുടെ വിളവ് T_6 ലും T_{14} ലും കൂടുതൽ രേഖപ്പെടുത്തി. ഫലപ്രദമായ രീതിയിൽ പ്രകാശസംശ്ലേഷണ ഉത്പന്നങ്ങളുടെ വിതരണം പയറുചെടിയുടെ വിത്തുസഞ്ചിയുടെ നീളവും വ്യാപ്തിയും T_6 ൽ വർദ്ധിപ്പിച്ചു.

വിത്ത് നട്ട് 25, 45, 60 ദിവസങ്ങളിൽ ഇല വിസ്തൃതി സൂചികയും സ്പാഡ് ഹരിതക യന്ത്ര തോതും T_6 ലും T_{14} ലും മികച്ചതായി കാണപ്പെട്ടു. കായിക വളർച്ചകാലഘട്ടത്തിലും പ്രത്യുത്പാദന കാലഘട്ടത്തിലും വർദ്ധിക്കുന്ന പ്രകാശസംശ്ലേഷണ നിരക്കും പ്രകാശസംശ്ലേഷണ ഉത്പന്നങ്ങളുടെ ശരിയായ വിതരണവും മൊത്തം ഉണങ്ങിയ വസ്തുക്കളുടെ ഉത്പാദനം, ആപേക്ഷിക വളർച്ചാ നിരക്ക്, വിള വളർച്ചാ നിരക്ക് എന്നിവ T_{14} ൽ കൂടുതലായി കാണപ്പെട്ടു. T_{14} ന് ശേഷം T_6 ഉം ഇതേ ഫലം പ്രകടമാക്കി. ഇലയിലെ ആപേക്ഷിക ജല ലഭ്യതയുടെ അളവ് വിത്തു പാകി 25 ദിവസങ്ങൾക്കു ശേഷം T_6 ൽ കൂടുതലായി കാണപ്പെട്ടു. അസ്ഥിര ഫ്ലൂറസെൻസിന്റെയും പരമാവധി ഫ്ലൂറസെൻസിന്റെയും അനുപാതം T_{14} ൽ കൂടുതലായി കാണപ്പെട്ടു. ഇത് T_6 നും T_{12} നും തുല്യമായിരുന്നു. വിത്തുപാകി 25 ദിവസങ്ങൾക്ക് ശേഷം ഫലപ്രദമായ ഊർജമാത്രയുടെ ഫലം പിഎസ് II ൽ T_6 ലും T_{14} ലും കൂടുതലായി രേഖപ്പെടുത്തി. ഇത് T_{10} നും T_{12} നും തുല്യമായിരുന്നു. 45 ദിവസങ്ങൾക്ക് ശേഷം T_{14} കൂടുതൽ മൂല്യം രേഖപ്പെടുത്തി. 60 ദിവസങ്ങൾക്ക് ശേഷം T_{14} കൂടുതൽ മൂല്യം രേഖപ്പെടുത്തി. വിത്തു പാകി 25 ആമത്തെ ദിവസം കോശങ്ങളിലൂടെയുള്ള ഇലക്ട്രോൺ പ്രവാഹത്തിന്റെ നിരക്ക് T_6 ലും T_{14} ലും മികച്ചതായി കാണപ്പെട്ടു. ഇത് T_8,

T_{10}, T_{12} എന്നിവക്ക് തുല്യമായിരുന്നു. 45 ഉം 60 ഉം ദിവസങ്ങൾ പിന്നിടുമ്പോൾ ഈ നിരക്ക് T_{14} നിൽ മികച്ചതായി രേഖപ്പെടുത്തി. വിത്ത് നട്ട് 25 ആം ദിവസം T_6 ലും T_{14} ലും കൂടുതൽ ഹരിതകത്തിന്റെ സാന്നിധ്യം കണ്ടെത്തി. 25, 45, 60 ദിവസങ്ങളിൽ ഇലകളിലെ പ്രോലിന്റെ സാന്നിധ്യം T_1 ൽ കൂടുതലായി കണ്ടെത്തി.

വിളവ് കണക്കിലെടുക്കുമ്പോൾ ശരാശരി വിത്ത് സഞ്ചികളുടെയും വിത്തുകളുടെയും എണ്ണം T_6 ലും T_{14} ലും കൂടുതലായി കാണപ്പെട്ടു. (100 ppm) സാലിസിലിക് അമ്ലം ഉപയോഗിച്ചുള്ള പ്രാഥമിക വിത്ത് പരിചരണവും ഇലകളിൽ തളിക്കലും വിളവ് 43 ശതമാനം വർദ്ധനവ് കണക്കാക്കുന്നു. അതേസമയം ബ്രസ്സിനൊലിടെ (0.3 ppm) ഉപയോഗിച്ചുള്ള പ്രാഥമിക വിത്തു പരിചരണവും ബ്രസ്സിനൊലിടെ (0.5 ppm) ഇലകളിൽ തളിക്കലും 42.11 ശതമാനം വിളവ് വർദ്ധനവ് രേഖപ്പെടുത്തി. പ്രാഥമിക വിത്ത് പരിചരണം ഇല്ലാതെ ബ്രസ്സിനൊലിടെ (0.5 ppm) ഇലകളിൽ തളിക്കുന്നത് 33.74 ശതമാനം വിത്തു വിളവ് കൂട്ടി. അതുപോലെ സാലിസിലിക് അമ്ലം (100 ppm) ഇലകളിൽ തളിക്കുന്നത് 33.29 ശതമാനം വിളവ് കൂട്ടി.

ഏറ്റവും ഉയർന്ന അറ്റാദായം ലഭിച്ചത് സാലിസിലിക് അമ്ലം (100 ppm) ഉപയോഗിച്ച് പ്രാഥമിക വിത്തു പരിചരണവും ഇലകളിൽ തളിക്കുകയും ചെയ്തപ്പോഴായിരുന്നു. ഇത് T_5, T_8 എന്നിവക്ക് ഒപ്പം നിൽക്കുന്നു. ബ്രാസ്സിനൊലിടെ പരിചരണം ചെടികളിൽ നിന്നുള്ള ആകെ വരുമാനം കൂട്ടുന്നെങ്കിലും ബ്രാസ്സിനൊലിഡിന്റെ വില കാരണം അറ്റാദായത്തിൽ കുറവ് വരുന്നു. ഏറ്റവും ഉയർന്ന വരവ് ചെലവ് അനുപാതം രേഖപ്പെടുത്തിയത് സാലിസിലിക് അമ്ലം നൽകുമ്പോഴാണ്. പ്രാഥമിക വിത്ത് പരിചരണം ഇല്ലാതെ ഇലകളിൽ ലായനി തളിക്കുന്നത് വഴി T_5 ൽ കൂടുതൽ അനുപാതം രേഖപ്പെടുത്തുന്നു.

സംയുക്ത രീതിയിൽ വളർച്ച ത്വരിത പദാർത്ഥങ്ങളായ സാലിസിലിക് അമ്ലം, ബ്രാസ്സിനൊലിടെ എന്നിവയുടെ ഉപയോഗം പയറുചെടിയിൽ വിവിധതരം വളർച്ചാ മാനദണ്ഡങ്ങളെയും വിളവിനെയും പരിപോഷിപ്പിക്കുന്നു. ഇതിൽ തന്നെ സാലിസിലിക് അമ്ലം (100 ppm) പ്രാഥമിക പരിചരണവും ഇലകളിൽ തളിക്കലും ബ്രാസ്സിനൊലിടെ (0.3 ppm) പ്രാഥമിക വിത്തു പരിചരണവും (0.5 ppm) ഇലകളിൽ തളിക്കലും മുന്തിയ പരിചരണ രീതിയായി പരിഗണിക്കാം.