"Optimization of Nano-micronutrients for phenology, growth, productivity and quality of *Withania somnifera* (L.) Dunal."

THESIS

Submitted to

Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur

In partial fulfillment of the requirements for

The Degree of

MASTER OF SCIENCE

In

AGRICULTURE

(PLANT PHYSIOLOGY)

By

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Jabalpur, Madhya Pradesh

2022

CERTIFICATE - I

This is to certify that the thesis entitled **"Optimization of Nano-micronutrients for phenology, growth, productivity and quality of *Withania somnifera* (L.) Dunal."** submitted in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE in AGRICULTURE** in the **Department of Plant Physiology** of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur is a record of the bonafide research work carried out by **Anee Jain** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instruction.

All the assistance and help received during the course of the investigations has been acknowledged by her.

Place: Jabalpur

Date: **(Dr.Gyanendra Tiwari)**

Chairman of the Advisory Committee

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Place: Jabalpur

Date**:** **(Dr.Gyanendra Tiwari)**

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Declaration and Undertaking by the Candidate

I, **Anee Jain** D/o **Ajit Kumar Jain** certify that the work embodied in thesis entitled **"Optimization of Nano-micronutrients for phenology, growth, productivity and quality of *Withania somnifera* (L.) Dunal."** is my own first hand bonafide work carried out by me under the guidance of **Dr. Gyanendra Tiwari** at Department of **Plant Physiology**, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) during 2020-22.

The matter embodied in the thesis has not been submitted for the award of any other degree/diploma. Due credit has been made to all the assistance and help.

I, undertake the complete responsibility that any act of misinterpretation, mistakes, and errors of fact are entirely of my own.

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ABBREVIATIONS

Legends	**:**	**Descriptions**
ANOVA	:	Analysis of Variance
CCI	:	Chlorophyll Content Index
CD	:	Critical Difference
CD at 5%	:	Significant at 5% Level of significance
cm	:	Centimeter
cm^2	:	Square centimeter
DAS	:	Days After Sowing
et al.	:	And others people
Eve.	:	Evening
Fig.	:	Figure
FW	:	Fresh weight
g	:	Gram
HI	:	Harvest Index
i.e.	:	That is
Kg	:	Kilogram
LAD	:	Leaf Area Duration
LAI	:	Leaf Area Index
m	:	Meter
m^2	:	Meter square
mg	:	Milligram
Morn.	:	Morning
MSS	:	Mean Sum of Square
MT	:	Million ton
No.	:	Number
$Plant^{-1}$	:	Per plant
ppm	:	Part per million

RF	:	Rainfall
SCMR	:	SPAD chlorophyll meter reading
SEm±	:	Standard error of mean
TDM	:	Total Dry Matter
Tem.max	:	Maximum temperature
Tem.min	:	Minimum temperature
var.	:	Variety
Viz.,	:	Namely
%	:	Percentage
^0C	:	Degree Celsius

INTRODUCTION

Ashwagandha [*Withania somnifera* (L.) Dunal.] is one of the most important medicinal plant belongs to family Solanaceae. It is also called as Indian Ginseng, poison gooseberry, winter cherry, Punir or Asgandh in local languages (Nigam and Kandalkar 1995).

Ashwagandha crop is small, erect, branched, evergreen, tomentose perennial woody shrub with an average height of 150-170 cm tall. Roots of this crop are stout fleshy, whitish brown in colour, and considered as the economical part due to higher withanolide content in it. Leaves are simple, elliptic ovate in shape, exstipulate, glabrous, flowers are greenish on lubrid-yellow, pedicellate umbellate cymes appears in cluster. Berries are small, bright orange red in color at full maturity which is enclosed in persistent calyx containing number of seeds. Seeds are small, smooth , yellowish in colour of 2 mm long, 1.5- 2 mm wide and 0.5 mm thick (Rao *et al*. 2012).

All the parts (like roots, leaves & seeds) are medicinally useful to treat various ailments. Although roots are main useful parts medicinally. The main active ingredients present in ashwagandha crop are alkaloids and withanolides. The chemical constituent Withanolide which is present in roots, leaves and in berries is used to cure many of the diseases.The roots of this crop are known to be as Rasayans and function as a tonic for longevity and vitality (Singh *et al.* 2010). Dried root powder is used to cure many diseases such as impotency, rheumatism, fever, cough, leucoderma, dropsy, stomach and lung inflammation, skin related disorders and some of the female related disorder (Bhaure *et al.* 2014).

Ashwagandha has been cultivated commercially as a rainfed crop in many states of India. Major leading states are Rajasthan, Punjab, Haryana, UP, Gujrat, Maharashtra and Madhya Pradesh (MP). *Withania somnifera* has been cultivated over an area of 10,780 ha with a production of 8429 tonnes. With the increasing annual demand its production also increased from 7,028 to 9,128 tonnes. This increase by 29.8 % the demand lead to an increase in area for its cultivation for higher and better quality produces (Tripathi *et al.* 1996). Out of total area, 4000 ha area under Ashwagandha cultivation is in the drier part especially in Manasa, Neemach, Jawad Tehsil of Madsaur District of Madhya Pradesh (Sharma, 2004).

It is cultivated as late rainy season (Aug-Sept) crop or as early winter season crop in India. It requires sandy loam soil with pH 7.5-8.0 (slightly alkaline) and a temperature ranging from 20^0C to 35^0C is best suited for its cultivation (Jat *et al.* 2015).

This crop mainly prefers organic manure as the source of nutrient, but it is not possible to fulfill its commercial demand, only through organic source. Therefore, supplementing the crop with different sources as well is very much essential. As a medicinal crop, too much use of agro-chemicals (especially chemical fertilizers) may affect product quality and may also detoriate soil physical, chemical and biological health.

To address the problem, there is an urgent need to develop an alternative nutrient management strategy with low concentration of nutrients which can improve crop growth, yield and quality parameters by enhancing the Nutrient use efficiency (NUE) of the crop.

Nano fertilizers is one the best alternative for fertilizers as they are encapsulated inside nano porous materials, coated with thin polymer film, or delivered as particle or emulsions of nano scales dimensions(Rai *et al.* 2012). These Nano fertilizers provide the major nutrients to the crop as per the requirement in a phased manner as it contains nutrients and growth promoters encapsulated in nano scale polymers. This provides the slow and target efficient release of nutrients to the crop during its life cycle (Tarafdar *et al.* 2014).

Micronutrient application is very important as our Indian soils are deficient in one or the other micronutrients. These micronutrients play major role in enhancing proper crop growth and maintaining soil health. Micronutrients also promote several metabolic activities of the crop such as photosynthesis, chlorophyll formation, resistance to plant diseases, enzyme activities for synthesis of primary and secondary metabolites (Adhikary *et al.* 2010). Therefore, application of recommended micronutrients is highly desirable for enhancing the crop growth and achieving the better quality produce.

Therefore, keeping the above mentioned facts in mind the present thesis work was designed in such a way to overcome the problem of micronutrient deficiency in soil by incorporating Nano micronutrients which will provide better yield and good quality product by enhancing the Nutrient use efficiency of the crop.

The present research work was undertaken considering the following two objectives:

1. To assess the effect of Nano micronutrient combination (Zn+Mn+Cu) on phenology and physiology of *Withania somnifera* (L.) Dunal.
2. To find out to effect of Nano micronutrient combination (Zn+Mn+Cu) on productivity and active ingredient content of the crop.

REVIEW OF LITERATURE

The present experiment on "**Optimization of Nano-micronutrients for phenology, growth, productivity and quality of *Withania somnifera* (L.) Dunal.**" was conducted in late *kharif* season 2021-2022. As Nanotechnology is recent technology therefore no or very little work reported earlier on nano-micronutrients. Review of work on nanotechnology and micronutrients application on growth and productivity of Withania and other solanaceous and medicinal crops was done under the following heads:

2.1 Effect of micronutrients on solanaceous crop.

2.2 Effect of micronutrients on medicinal crop.

2.3 Effect of nano-micronutrients on solanaceous and medicinal crop.

2.1 Effect of micronutrients on solanaceous crop

2.1.1 Effect of micronutrients on growth parameters

Mallik and Muthukrishnan (1980) applied Zn, Fe, Cu and Mn to tomato, as foliar sprays at 3000 or 5000 ppm or soil treatments @ 5, 10 or 20 ppm. They observed that the flowering tended to be extended by the micronutrient treatments and the number of flowers also tended to be increased as compared to control by all treatments except foliar Cu spray.

Jana and Jahangir Kabir (1987) observed that individual foliar spray of boron. molybdenum, iron, zinc, manganese and magnesium each at 0.2% concentration increased the plant height 56.8, 56.3, 52.1, 57.5, 54.8 and 45.7 cm, respectively compared to the same elements applied at 0.1% concentration and also observed that combined foliar spray of B+ Cu + Mo +Fe+Zn+ Mn+ Mg at 0.1% concentration resulted in maximum height of 61.0 cm in French bean.

Singh *et al*. (1989) noted highest number of branches as 7.52 in plant receiving 20 kg/ha $ZnSO_4$, 0.5 % $ZnSO_4$, as foliar spray, whereas it was minimum as 3.25 in control in chilli cv. Local Faizabad'. These may be that due to the fact that zinc act as an enzyme activator and increases the auxin activity which plays essential role in the growth and development of plant.

Dod et al. (1989) observed that foliar application of 0.2 % $ZnSO_4$, 0.2 % $MnSO_4$, 0.2% $CuSO_4$, and NAA at 50 or 100 ppm, 2,4,5-T at 50 or 100 ppm to capsicum cultivar Jwala once at full bloom stage and second after 21 days, resulted in increase in plant height (84.6 cm).

Hussani *et al*. (1989) worked on chilli to see the response of foliar spray of Zn, B, iron, individually at 0.1% that resulted in increased plant height 56 cm, 62 cm and 60 cm, respectively also combined foliar spray of zinc, boron and iron at 0.1% each increases plant height (62 cm) compared to the control.

Romheld and Marscher (1991) revealed that zinc is an essential component and activator of many enzymes which take part in auxin biosynthesis and photosynthesis and therefore plays a major role in plant growth. Increase in plant height is also attributed due to the role of zinc in auxin synthesis and association of boron for development of cell wall and cell differentiation that plays major role in root and shoots growth in plants. Application of zinc at absolute concentration provides better root system for the plant for absorption of water and other nutrients dissolved in it.

Kumbhar and Deshmukh (1993) recorded that soil application of 120 kg ferrous sulphate/ha to tomato cv. Rupali, resulted in maximum plant height (68.28 cm), while it was lowest (47.0 cm) in untreated control. They also revealed that among the other micronutrients (Zn, Cu, Fe, Mn), soil application of Zn was found to be more effective in promoting the growth and development of tomato plants. This may be because zinc is an enzyme activator and it involves itself in auxin synthesis, which is one of the growth regulating substances in plants.

Ravichandran *et al*.(1995) observed that soil application of $ZnSO_4$ (25 kg/ha) and 0.5 % Zn as foliar spray recorded highest plant height (142 cm) as compared to control (118 cm) in Brinjal (Var. Annamalai).

Bose and Tripathi (1996) noticed the maximum plant height (81.56 cm) of tomato cv. 'Pusa Ruby' as a result of combined foliar application of micronutrients (Zn, Mn, Fe, B).

Bose and Tripathi (1996) recorded the highest number of branches/plant (19) after combined application of micronutrients (Zn, Mn, Fe and B) as compared to control in tomato cv. 'Pusa Ruby''.

Yadav *et al*. (2001) revealed the effect of different concentrations of zinc and boron on the vegetative growth, flowering and fruiting of tomato. The treatment consist of five levels of zinc (0, 2.5, 5.0, 7.50 and 1.00ppm) and four levels of boron (0, 0.5, 0.75 and 1.00ppm) as soil application as well as 0.5% zinc and 0.3% boron as foliar application resulted in the highest values for secondary branches, leaf area, fresh weight, fruit length, fruit breadth and fruit number were obtained with the application of 7.5ppm zinc and 1.0ppm boron.

Baloch *et al*. (2008) revealed that foliar spray of HiGrow, which is a commercial foliar fertilizer, consists of various macro (N, P, K, Ca and Mg) and micronutrients (Fe, Mn, B, Cu and Mo) sprayed at the concentration of 8 ml/L recorded the highest plant height (68 cm) in chilli.

Kumar *et al*. (2008) observed that the growth parameters of potato crop were significantly influenced by the application of different micronutrients. The highest plant height (45.8 and 62.9 cm), number of shoots hill (2.94 and 4.39) and leaf area (40.7 and 55.0 cm^2) were obtained at 45 and 60 days after sowing, respectively, as seed tubers was treated with 0.05% $ZnSO_4$.Different concentrations of zinc sulphate (0.05, 0.10 and 0.15%) used for treating potato seed tubers showed significant effect on growth and development of potato plant and highest vegetative growth was seen at 0.10% zinc sulphate.

Rahman *et al*. (2011) while working on potato crop observed that due to deficiency of zinc, performance and quality of potato decreased. While the highest number of leaves/ plant and main stem/hill (8.36) was produced by application of Zn at 4 kg/ha followed by 3 kg and 5 kg/ha, while the lowest number of main stem (7.28) was found in control. Foliar application of six active yeast extract (0, 1, 2, 3, 4 and 5 g/L) with four zinc fertilization rates (0, 100, 200 and 300 ppm) significantly enhanced vegetative growth of potato cv. Valor. Increasing concentration of zinc up to 300 ppm along with 5g/L yeast extract increased the vegetative growth characters, i.e. plant length, stem and leaves number per plant, leaf area per plant and fresh and dry weight of whole plant.

Khasti and Rana (2012) noted that foliar feeding of Zn @ 4 mg remarkably improved vegetative growth parameters, total yield and quality contents in bulb tissues in onion compared to other micronutrients. Foliar spraying of Zn gave the superior performance in all measured parameters than other treatments.

Haleema *et al*. (2013) observed increased number of leaves plant^{-1} (177), with the combined foliar application of calcium (0.6 %), boron (0.25 %) and zinc (0.5 %) and can be used to improve growth and fruit production in tomato.

Ali *et al*. (2013) examined the possible effect of some macro and micro nutrients with different concentration levels as a foliar application on the vegetative growth, flowering, and yield of tomato cv 'Roma'. The important parameters encompassed in the study were plant height (cm), number of leaves plant^{-1}, leaf length (cm), days to flowering, , small fruits plant^{-1}, medium fruits plant^{-1}, large fruits plant^{-1}, length and width of fruit (cm), fruit weight (g), fruit yield plant^{-1} (kg), yield plot^{-1} (kg) and yield hectare^{-1}. Though all the treatments showed a positive effect on growth, flowering, and yield but, T_5 (nitrogen 5.5 g/100 ml + Boron 5 g/100mL + Zinc 5 g/mL) and T3 (Boron 5 g/mL) showed most significant influence on all parameters under study as compared to T_1 (control). Therefore, it can be said that foliar application is an appropriate way to feed the tomato crop to enhance the growth, flowering and marketable yield.

Naga *et al*. (2013) worked on the response of foliar application of micronutrients on vegetative and reproductive growth attributes, in two varieties of tomato viz Utkal Kumari and Utkal Raja. The following treatments consist of boron, zinc, molybdenum, copper, iron, manganese, mixture of all and control. All the Micronutrients except manganese at 50ppm were applied at 100ppm in three sprays at an interval of ten days starting from 30 days after transplanting. All treatments resulted in enhance plant growth characteristics such as plant height, number of primary branches, compound leaves, tender and mature fruits per plant in both the varieties among all application of micronutrients mixture showed the maximum effect.

Kalroo *et al*. (2014) recorded that the foliar zinc was applied at the concentrations of 1, 2, 3, 4 and 5 ml/L water and control was maintained to check the performance resulted that all the growth and green chillies production traits were significantly influenced under foliar zinc application at different concentrations. The highest zinc concentration of 5 ml/L water resulted 85.66 cm plant height, 56.33 days to flower emergence, 77 cm plant spread, 13.00 branches plant^{-1}, 481.33 fruits plant^{-1}, fruit length 5.50 cm, fresh fruit yield 705 g plant^{-1}and 16.350 tons fruit yield ha^{-1}.

Jobori and Hadithy (2014) revealed that foliar application of Zn, Mn, Fe and Cu increased all plant characteristics relating to yield components, i.e., number of tuber per plant, yield and dry matter % of potato crop. Application of full micronutrients mixture (330 g $ZnSO_4$+ 330 g $MnSO_4$+ 150 g $FeSO_4$+ 80 g $CuSO_4$ dissolved in 1000 liter water) maximized yield to 22.89 ton/ha. However, application of half concentration of micronutrients mixture decreased yield of potato tuber to 20.01 ton/ha. Application of micronutrients mixture during flowering stage increased tuber yield in compare with application of micronutrient mixture at10 days before flowering and 20 days after flowering up to 13.74 and 16.18%, respectively. Application of full concentration of micronutrient mixture during flowering increased number of tubers (10.67) per plant, weight of tubers (82.02 g/plant) and dry matter 22.91 % in potato tubers.

Tawab *et al*. (2015) recorded that the four levels of zinc (0, 0.1, 0.2, and 0.3%) were applied to three brinjal cultivars (Purple, Shimla, Shamli). Both cultivars and zinc levels proved significantly different among growth parameters viz. Plant height, number of leaves per plant, numbers of fruits per plant, fruit weight and total yield were significantly increased by zinc levels: Maximum plant height (131.89cm) , number of leaves per plant (437.78), number of fruits per plant (9.00), fruit weight (280.11g) and total yield (15.33 t/ha) were recorded for plants treated with 0.2% zinc, while least number of leaves per plant (231.33), number of fruits per plant (5.33), fruit weight (143.89 g) and total yield (4.51 t/ha) were recorded in control treatments.

Ali *et al*. (2015) studied the effect of foliar application of zinc and boron (T_0:control; T_1: 25-ppm $ZnSO_4$ (Zinc Sulphate); T_2: 25-ppm $ZnSO_4$ H_3BO_3 (Boric Acid) and T_3: 12.5-ppm $ZnSO_4$ 12.5-ppm H_3BO_3) in tomato resulted in maximum plant height (106.9 cm), number of leaves (68.9/plant), leaf area (48.2 cm2), number of branches (11.9/plant), number of clusters (21.6/plant), were found from foliar application of 12.5-ppm $ZnSO_4$+ 12.5-ppm H_3BO_3) while minimum from control. Early flowering (49.3 days) were also found from foliar application of 12.5 ppm $ZnSO_4$-12.5 ppm H_3BO_3.

Harris and Mathuma (2015) revealed that foliar application of Zn alone at 250 ppm resulted in the maximum plant height, total dry weight, number and fresh weight of fruits/ plant in tomato,while foliar application of B at 250 ppm increased dry weight of leaves/ plant and dry weight of stem/ plant, and dry weight of roots/plant were high in both B at 250 ppm and Zn at 150 ppm. In all parameters, the lowest performance was seen in the control treatment.

2.1.2 Effect of micronutrients on yield parameters

Bid *et al*. (1992) noticed that zinc sulphate and copper sulphate at 10 and 20 kg/ha, respectively decreased zinc and copper deficiency symptoms and significantly increased the yield of brinjal.

Ravichandran *et al*. (1995) revealed the highest fruit yield of brinjal with soil application of 25 kg ha' zinc sulphate along with 0.5 percent foliar spray of $ZnSO_4$, at 30 days after transplanting on zinc deficient silt clay loam soil at Annamalainagar in Tamil Nadu

Bose and Tripathi (1996) observed that after combined application of Zn, Mn, Fe and B at 30 and 60 days after transplanting resulted in highest yield per plant (1.407 kg) in tomato cv. Pusa Ruby.

Raj *et al*. (2001) noted significant increase in yield, zinc and iron content of egg plant fruits with the application of zinc and iron either through soil or foliar spray. Among all other the treatments, soil application of $ZnSO_4$ 12.5 kg/ha along with three sprays of $ZnSO_4$0.2% and $FeSO_4$ 0.5% at weekly interval at later stages recorded highest fruit yield of 37.7 t/ha in brinjal cv. Bhagyamathi.

Hatwar *et al*. (2003) reported that combined foliar application of zinc, iron and boron at one per cent was found effective in respect of yield per plant (497 g) as compared to other treatments in chilli.

Raghav and Singh (2003) observed that potato yield and yield attributing characters were enhanced with the basal as well as foliar application of zinc sulphate. The highest number of small tubers was produced with zinc at 2 kg/ha, whereas, maximum weight of small tubers was obtained with zinc at 5 kg/ha (applied during 30 and 45 days after planting). However, the highest number and weight of medium and large tubers were robserved with basal application of zinc at 8 and 10 kg/ha, respectively. The maximum tuber yield (32.21 t/ha) was attained with soil application with zinc sulphate @ 8 kg/ha as compared to foliar application of zinc sulphate in potato

Puzina (2004) observed that potato-tuber treated with 3 mM zinc sulphate, 8 mM boric acid and water (control) remarkably enchanced the potato tuber yield under greenhouse condition. Zinc sulphate treated crop gave highest chlorophyll content (0-40 mg/g), fresh leaf weight (83.5 g/plant), fresh haulms weight (106.7 g/plant) and fresh root weight (11.3g/plant) as compared to boric acid and water (control). Moreover , different concentration of zinc sulphate significantly effects the vegetative growth of potato plant.

Karuppaiah (2005) reported that foliar application of borax (0.5 %) at 35, 50 and 65 DAT was seem to be best in terms of number of flowers per plant, number of productive flowers per plant, number of fruits per plant, individual fruit weight and yield (32.15 t ha^{-1}), followed by copper sulphate (0.5%) and zinc sulphate (0.5%) sprayed at 35, 50 and 65 DAT in brinjal cv. Annamalai.

Patil *et al*. (2008) observed that the application of boric acid @of 100ppm resulted in maximum number of primary branches (18.30), yield per plant (2.07kg) and fruit yield (30.50 t/ha) in tomato. Followed by best treatment was the mixture of micronutrients (Bo, Zn, Mn and Fe@ 100ppm and Mo@ 50ppm) recording fruit yield of 27.98 t/ha and differed significantly from other treatments.

Smitha and Ukkund (2008) revealed the effect of foliar application of micronutrients on growth and yield of tomato.It has been observed that out of nine different treatments, the application of micronutrients (Bo, Zn, Mn and Fe@ 100 ppm and Mo @ 50 ppm) recorded highest fruit yield of 27.98 t/ha and differed significantly from other treatments.

Ahmed *et al*. (2011) revealed that foliar spray of 300 ppm zinc fertilizers along with 5 g/L yeast extract significantly influenced the yield and quality of potato tuber. The maximum total tuber yield (18.769 and 19.055 ton/fed.) and dry matter (21.3 and 21.5%) was found with foliar spray of yeast at 5 g/L combined with foliar spray of zinc at 300 ppm in both seasons, respectively.

Kumar *et al*. (2012) found that the foliar application of micronutrients solution proved useful in case of mean values of transverse length, polar length, pericarp thickness, locule number, fruit weight and density and yield of tomato variety, Rupali. Following three combined foliar applications of B, Zn, Cu Fe and Mn each @ 100 ppm and Mo@50 ppm at 10 days interval starting from 40 DAT of tomato enhanced significantly and resulted in maximum fruit yield.

Singh *et al*. (2014) observed that the combined foliar application of Zn 100 ppm, B 100 ppm, Mo 50 ppm, Fe 100 ppm, Cu 100 ppm and Mn 100 ppm with the soil application of 120kg N, 60 kg P_2O_5, 80 kg K_2O, 10 t FYM 25 kg S and 5 kg Azotobacter ha^{-1} resulted in maximum plant height (103.57 cm), number of branches plant (11.33), number of compound leaves plant (30.53), weight of marketable fruits plant (1.80 kg) and fruit yield of tomato hectare' (308.73 q) and minimum weight of unmarketable fruits plant (0.29 kg).

Jam *et al.* (2015) noted that application of 40 ppm iron and 20 ppm zinc produced higher tuber number but the tuber yield (481 q/ha) and skin thickness were resulted maximum at 20 ppm iron and 80 ppm zinc.

Jawad (2016) revealed that the effect of foliar application of zinc and manganese on potato production and observed that application of combined solution of zinc and manganese increased mean weight of potato tubers to 94.03 g per tuber which was 65% higher, tuber yield 921.90 g per plant and total tubers yield to 46.10 tonnes per ha. The interaction effect (spray of Zn+Mn x application date) was significant on yield parameters of potato such as, mean weight of potato tuber, tuber yield per plant and total tubers yield significantly increased with the combined application of zinc and manganese solution at vegetative growth stage which were 98.87 g tuber 941 40 g per plant and 47.07 tonnes per ha, respectively.

Singh *et al*. (2017) worked on sweet potato to study the effect of different levels of potassium and zinc on growth, yield and economics of sweet potato, and found that foliar application of zinc (30 ppm) significantly increased the number of tubers per plant (4.18).

2.1.3 Effect of micronutrients on physiological and biophysical traits

Elbaky *et al*. (2006) reported that foliar application of zinc 30 ppm had a positive effect on vegetative growth of sweet potato plants as resulted in highest number of leaves (374.25) and leaf area per plant 1.22 m^2.

Kumar *et al*.(2008) worked using different micronutrients such as zinc sulphate (0.05%), copper sulphate (0.05 %) and magnesium sulphate (0.05 %) along with a control treatment and found maximum leaf area (40.7 $cm^2$and 55.0 cm^2) by combined foliar application of zinc sulphate, copper sulphate and magnesium sulphate along with recommended dose of fertilizers.

Salam *et al*. (2011) observed highest chlorophyll-a (420 mg/100g), chlorophyll-b (610 mg/100g) with the combined application of 2.5 kg boron, 6 kg zinc and 20 t cow dung per hectare.

Razek *et al*. (2013) noticed that spraying Fe+ Zn+ Mn @ 4 g/ha increased chemical constituent, chlorophyll-a, chlorophyll-h, significantly in all treatments with micronutrients in faba bean.

Banerjee *et al*. (2016) noted that application of 4.5 kg $Znha^{-1}$ observed higher Leaf area index (2.98) in potato.

2.1.4 Effect of micronutrients on quality attributes

Bhatt and Srivastava (2005) observed the response of foliar application of micronutrients, viz boron, zinc, molybdenum, copper, iron, manganese, mixture of all on physical characteristics and quality attributes of tomato fruits. The application of mixture of micronutrients resulted in maximum fruit density (1, 10 g/cc) and average fruit weight (49.83 g)

Mousavi *et al*. (2007) observed that application of zinc and manganese increased all the yield and quality attributing characteristics of potato, i.e., tuber yield per plant, dry matter percentage, specific weight, protein and starch content of tuber. Application of zinc at 8 ppm increased the yield to 34.70 q/ha while application of manganese at 4 ppm increased yield to 338.66 q/ha. Maximum number of tuber per plant (12.00), tuber weight (79.16 g) and yield (389.50 q/ha) were obtained with the foliar application of mixture solution of 8 ppm zinc and 4 ppm manganese on potato crop. Application of 8 ppm zinc and 4 ppm manganese together also increased the protein (36.5%) and starch content (14.9%) of potato tuber.

Kumari (2012) conducted an experiment to assess the effects of micronutrients which are boron, zinc, molybdenum, copper, iron, manganese, mixture of all and multiples through foliar application on quality of fruit and seed in tomato. Three sprays of each at 100 ppm were applied at 10 days interval starting from 30 days after transplanting observed variation in total soluble solids which was remarkable also maximum increase in vitamin C content of tomato fruits (25.27 mg/100 g) was observed with the application of zinc which accounted for an increase of 36.89 percent as compared to 18.46 mg/100 g in control.

Saravaiya *et al*. (2014) study the effect of foliar application of micronutrients in tomato (Lycopersicon esculentum Mill.) cv. Gujarat Tomato 2. They observed result clearly showing that the yield obtained with treatment T7 (NPK-mixture of all nutrients) had significantly maximum fresh weight of plants (25.65 t ha^{-1}), number of fruits $plant^{-1}$ (34.26), fruit length (5.52 cm), fruit diameter (4.64 cm), fruit volume (67.53 cm3). Single fruit weight (49.20 g), fruit weight per plant (1.68 kg fruit yield ha^{-1} (46.781) and marketable fruit yield ha^{-1} (45.62 t). This treatment also had maximum net retain (1, 66,757 Rs/ ha) and BC Ratio 2.72 :1 out all other treatments than over control.

Parmar *et al*. (2016) recorded that yield and quality traits were significantly influenced with the foliar application of 15 ppm zinc and 6 ppm manganese in potato. As the highest tuber yield per plant (610.43 g), tuber yield per hectare (417.61 q) and quality parameters viz., reducing sugar (3.53%), non reducing sugar (4.33%), total sugars (7.86%) and protein

(2.60%) were obtained in crop which was sprayed with 15 ppm zinc and 6 ppm manganese.

2.2 Effect of micronutrients on medicinal plants

2.2.1 Effect of micronutrients on growth and yield attributes

Maurya (1990) examined the effect of micronutrients on the yield of coriander (Coriandrum sativum L.) cv. Rajendra Swati. In this experiment, Rajendra Swati was grown with three foliar sprays (at 25, 37 and 49 days after sowing) of water; 0.5 or 1 per cent Tracel-2; 0.25 per cent borax, ammonium molybdate or $CuSO_4.5\ H_2O$; 0.5 per cent $ZnSO_4.7H_2O$ or $MnSO_4.4\ H_2O$; 2 per cent $FeSO_4.\ H_2O$ + 0.2 per cent citric acid. The findings of the study observed that $CuSO_4$ gave the highest yield in all cases.

Khattab and Omer (1999) revealed the effect of application of higher doses of fertilizers with microelements on growth, yield and chemical composition of caraway, anise, coriander and fennel plants. The plants were grown in clay loamy. Soil and foliar spray with five treatments (0, 100, 200, 300 and 400 ppm) of the combined fertilizer (Pholaz D) which contained Fe, Zn and Mn. The plant yield maximum with application of 100 ppm microelements.

Kanujia (2006) conducted an experiment to study the effect of micronutrients on growth and yield of cabbage var. "Golden Acre during Rabi 2001 and Kharif 2002. The experiment was laid out in RBD and each treatment replicated thrice. The micronutrient treatments included boron, manganese, iron, copper, molybdenum, zinc, mixture of all and multiplex, including a control. All the treatments were applied @ 100 ppm as foliar spray 40, 50 and 60 days after transplantation. The study revealed that the foliar application of Zn@ 100 ppm resulted in maximum head weight and yield.

Fatima *et al*. (2007) studied the effect of newly developed fertilizer named fertilizer Fritz (composed of Fe, Mn, Zn, Cu and B) on Nigella sativa, Coriandrum sativum and Ptychotis ajowan. Crops were grown in Randomized block design with two treatments (T1-10 g Fritz and T2- control) and three replications. They observed that micronutrient treated plot showed positive impact on the yield of plants as a whole and on their constitutive parts as compared to control.

Kalidasu *et al*. (2008) revealed the influence of micronutrients on growth and yield of coriander (Coriandrum sativum) in rainfed vertisols. The field experiment was conducted during 2004-05 Rabi season at Regional Agricultural Research Station. They reported that the application of $ZnSO_4$+ $FeSO_4$+ $CuSO_4$ + $MnSO_4$, all at 0.5 per cent, recorded high yield of coriander (940 kg ha^{-1}) followed by $FeSO_4$, 0.5 per cent (927 kg ha^{-1}) and $ZnSO_4$, 0.5 per cent (922 kg ha^{-1}) which were at par with each other and superior over control (801 kg ha^{-1}).

Pariari *et al*. (2009) showed that foliar application of boron @0.1% and zinc@ 0.2% twice had been found to be effective in enhancing most of the yield attributes and seed yield of fenugreek. It is also seen that lower concentration is more effective than higher concentration.

Nadergoli *et al*. (2011) revealed that micronutrients involved zinc sulphate and manganese sulphate and method and time of application involved control, soil application, foliar application at shooting stage, at flowering stage, at podding stage, at shooting and flowering stages, at shooting and podding stages of common bean. The result showed that the highest stem height, number of seeds per pods, number of pods per plant, shilling percentage, yield and harvest index wereobserved by foliar application at shooting, flowering and podding stages, respectively also seen that application of zinc sulphate produced the highest number of unripe seeds. The highest leaf area has been observed at foliar application of manganese sulphate at podding stage.

Shabanzadesh and Galavi (2011) observed that the foliar application of Micronutrients(B, Zn, Fe) significantly affected plant height, capsule number per plant, number of seeds per plant and capsule, grain and biological yield and harvest index. The differences amongst micronutrients spraying treatments and the control were significant for all above mentioned traits. Foliar application with the mixture of three micronutrients (B,Zn and Fe) resulted in the greatest grain yield of black cumin.

Jakhar *et al*. (2013) conducted an experiment on the effect of zinc (Z_0-control, Z_1 -10mg & Z_2- 20 mg/ kg soil) on growth and yield of fenugreek (Trigonella foenumgraecum L..) at Jobner (Rajasthan). They noticed increase in the yield of fenugreek with increase in the levels of Zn.

Diana and Nehru (2014) conducted the field experiment to find out the role of micronutrients ($FeSO_4$, $ZnSO_4$, $CuSO_4$ and $MnSO_4$) on yield of coriander and revealed that the foliar application of 0.5 per cent iron and zinc resulted in maximum yield as compared to other treatments.

Mehrab (2014) observed the effect of foliar application of micronutrients (Cu, Mn, Fe and Zn) at 0, 200 and 400 ppm concentration, on growth, yield and essential oil content of Thyme (Thymus vulgaris L.) and found that the application of 200 ppm of Cu, Mn, Fe and Zn resulted in highest yield of Thyme.

Ahmed *et al*. (2015) conducted an experiment on foliar application of micronutrients reported that the foliar application of micronutrient mixture improved the yield of fenugreek.

2.2.2 Effect of micronutrients on physiological and quality parameters

Yongqing and Shiming (1997) studied the effect of zinc fertilization on quality of broccoli. The experiment revealed that application of $ZnSO_4$, with basal dose NPK fertilization significantly increased the chlorophyll content of broccoli.

Sharma (2012) assessed an experiment to study the effect of foliar spray of micronutrients on quality of broccoli. He observed highest chlorophyll content (24.82 mg 100 gm^{-1}) in the broccoli with the application of 0.45 per cent zinc sulphate.

Lalelou et al. (2013) observed the effect of various concentrations of zinc on chlorophyll content in pumpkin and found that higher zinc concentrations decreased the chlorophyll pigments in pumpkin.

Kazemi (2013) conducted a field trial to study the effects of foliar application of zinc (15, 30 and 50 mg L^{-1}) and iron (50 and 100 mg L^{-1}) and their combination on quality of cucumber and reported that the highest chlorophyll content was observed by the application of Zn @ 50 mg L^{-1} and Fe@ 100 mg L^{-1}.

Diana and Nehru (2014) assessed an experiment to find out the role of micronutrients ($FeSO_4$, $ZnSO_4$, $CuSO_4$ and $MnSO_4$) on quality of coriander and noted that the foliar application of 0.5 per cent iron and zinc resulted in improvement of quality compared to other treatments.

Zahara *et al*, (2018) found that foliar application of copper sulphate and copper nanoparticles applied to the leaves had a substantial impact on chlorophyll a, b, and total chlorophyll, dry matter yield, essential oil percentage, stem length, and essential oil production.Copper nanoparticles fertilizer at concentrations of 0.5, 1.0, and 1.5 g/L significantly influenced all types of chlorophyll content by 25, 35, and 45 percent, respectively, and essential oil percentage by 10, 20, and 23 percent, compared to the control. By increasing concentrations of copper nanoparticles (1.5 g/L) resulted in higher values of chlorophyll a, b, and total chlorophyll content, in terms of yield and morphological features, copper sulphate (0.5 g/L) boosted stem number, aerial dry yields, and essential oil yield by 25, 58, and 61 percent, respectively, over control.

2.3 Effect of nano-micronutrients on solanaceous and medicinal crop

Singh *et al*. (2017) reviewed the importance of nanofertilizers in increasing NUE of crops.They narrated that nanofertilizers are the important tools in agriculture to improve crop growth,yield and quality parameters with increase NUE, reduce wastage of fertilizers and cost of cultivation.

Nanofertilizers also provide more surface area for different metabolic reactions in the plant.

Kaur *et al.* (2018) reviewed the foliar application of Zinc and Manganese and their effect on yield and quality parameters of potato. The experiment was conducted in randomized complete block design with 9 treatment of different combination of Zn and Mn and one control. They observed that 10ppm Zn and 10ppm Mn showed significant impact on plant height, number of branches, tuber yield and also some of the quality traits such as protein and total soluble solids.

Mijwel and Abbas (2019) assessed the effect of genotype and nanofertilizers on some traits and yield of Potato. They stated that application of nanofertilizers resulted in increase in starch content in the tubers.

Mishra *et al*. (2020) assessed the effect of Nano fertilizers on growth, yield and economics of tomato var Arka Rakshak. They observed that applying 50% N +100%PK+50% Zn + 1st spray Nano N + 2nd spray Nano Zn + 3rd spray Nano Cu @4ml /L produced maximum plant height , more number of branches , increase in fruit weight and maximum yield is als ofo obtained. It has also been seen maximum benefit cost ratio and gross income is also obtained by the application 50% N +100%PK+50% Zn + 1st spray Nano N + 2nd spray Nano Zn + 3rd spray Nano Cu @4ml /L.

Mandal and Lalrinchhani(2021)reviewed on nanofertilizer and its application in horticulture. They reported that regular release of nutrients by nanofertilizer may help in augmenting nutrient use efficiency[NUE], increase in fruit yield and quality of various horticultural crops.

Seleiman *et al*. (2021) reviewed that nanofertilization is an emerging fertilization technique. They reported that nanofertilizers have the potential to promote sustainable agriculture and increase overall crop productivity, mainly by increasing the NUE of field and greenhouse crops.

Kanwal *et al.* (2022) observed the agricultural application of synthesized ZnS Nanoparticles for the development of tomato crop. They sprayed zinc sulfide ZnS nanoparticles four times at 15 days intervals and found effective as treated plants showed significant increase in weight and quality of produce.

Batool *et al.* (2022) reported qualitative and quantitative traits of sweet pepper as influenced by copper nanoparticles. They found enchancement in growth and quality parameters of sweet pepper by the foliar application of Cu nanoparticles of different concentrations among which 20mg/L CuNPs give best result as maximum growth , more number of fruits is obtained.

MATERIALS AND METHODS

The present research work entitled "**Optimization of Nano-micronutrients for phenology, growth, productivity and quality of *Withania somnifera* (L.) Dunal.**" was conducted during the late *Kharif* session [2021-2022] taking seven different treatments of six different concentration of Nanocombi (Zn+Mn+Cu) and one control treatment in a Randomized Block Design with 3 Replications at Experimental field of AICRP on Medicinal and Aromatic Plants JNKVV , Jabalpur, MP. The details of the materials and the methods used to conduct this experiment are listed below:

3.1. Experimental site

The experiment was conducted during Kharif season of 2021-2022 at Experimental field of AICRP on Medicinal and Aromatic Plants JNKVV, Jabalpur, MP. The topography of the experimental field was uniform and plain with good irrigation facilities.

3.2 Climate

Jabalpur is situated at 23'90.N latitude and 79'58.E longitude at an altitude of 411.78 meter over the mean ocean level. The climate of Jabalpur region is typically subtropical having hot dry summers and cool dry winters. The average annual rainfall is 1208 mm which is mostly received during June to October from south-west monsoon. The average maximum temperature is 38.1^0C and minimum temperature is 4.8^0C.

The meteorological parameter during the crop season such as minimum maximum temperature, sunshine hours, rainfall number of rainy days and relative humidity were recorded at Agro meteorological Department, College of Agricultural Engineering, JNKVV, Jabalpur and presented in Table 3.1 and depicted in Fig 3.1

Table 3.1: Weekly weather data during the experimental period late *Kharif* season (August 2021 to March 2022) (AICRPAM, Jabalpur)

Months	weeks	TEMP (·C)		RH (%)		Sun Shine hrs.	Rainfall (mm)	No. of Rainy days
		Max.	Min.	Mor.	Eve.			
August	32	28.5	24.2	90	79	0.5	11.1	2
	33	32.6	24.7	89	71	4.9	42.3	3
	34	30.7	24.7	87	67	2.6	2.2	0
	35	31.6	24.5	86	68	5.1	2.5	0
September	36	31.4	24.1	91	78	4.1	35.7	4
	37	30	23.8	93	81	2.3	100.8	4
	38	30.9	23.6	89	68	4.7	16.3	2
	39	31.9	24.1	88	62	5.6	3.8	1
	40	32.9	23.7	84	58	8.2	0	0
October	41	33.3	20	84	48	8.6	0	0
	42	31.9	19.9	88	48	8.1	67	2
	43	30.7	16	87	41	8.3	0	0
	44	28.7	11	84	33	9	0	0
	45	28.7	10.5	84	34	7.8	0	0
November	46	27.6	12.8	85	45	4.9	0	0
	47	29.1	15	87	46	4.3	1.8	0
	48	27.1	8.9	86	33	7.7	0	0
	49	26	12.3	89	53	3.7	0	0
December	50	25.4	8.9	86	41	5.4	0	0
	51	23.8	4.8	76	31	7.8	0	0
	52	23.4	8.9	88	58	5.6	23.8	2
January	1	23.2	8.6	85	50	7	0	0
	2	21.9	12.9	93	69	2.6	10.4	2
	3	20.8	7	91	57	5.2	0	0
	4	21.6	7.1	88	52	7	5.7	1
	5	23	3.9	75	33	10	0	0
February	6	24.4	7.4	81	39	7.5	4.4	1
	7	24.7	8	82	31	9.5	0	0
	8	29	11.4	83	30	8.7	0.2	0
	9	29.1	13.4	83	41	8.3	7	1
March	10	30.3	12.6	81	31	7.7	0	0
	11	34.5	14.2	80	25	9	0	0
	12	36.8	18.2	75	20	7.5	0	0
	13	38.1	15.8	68	14	7.5	0	0

Source: **Department of Agro meteorology, College of Agricultural Engineering, JNKVV, Jabalpur,(Madhya Pradesh).**

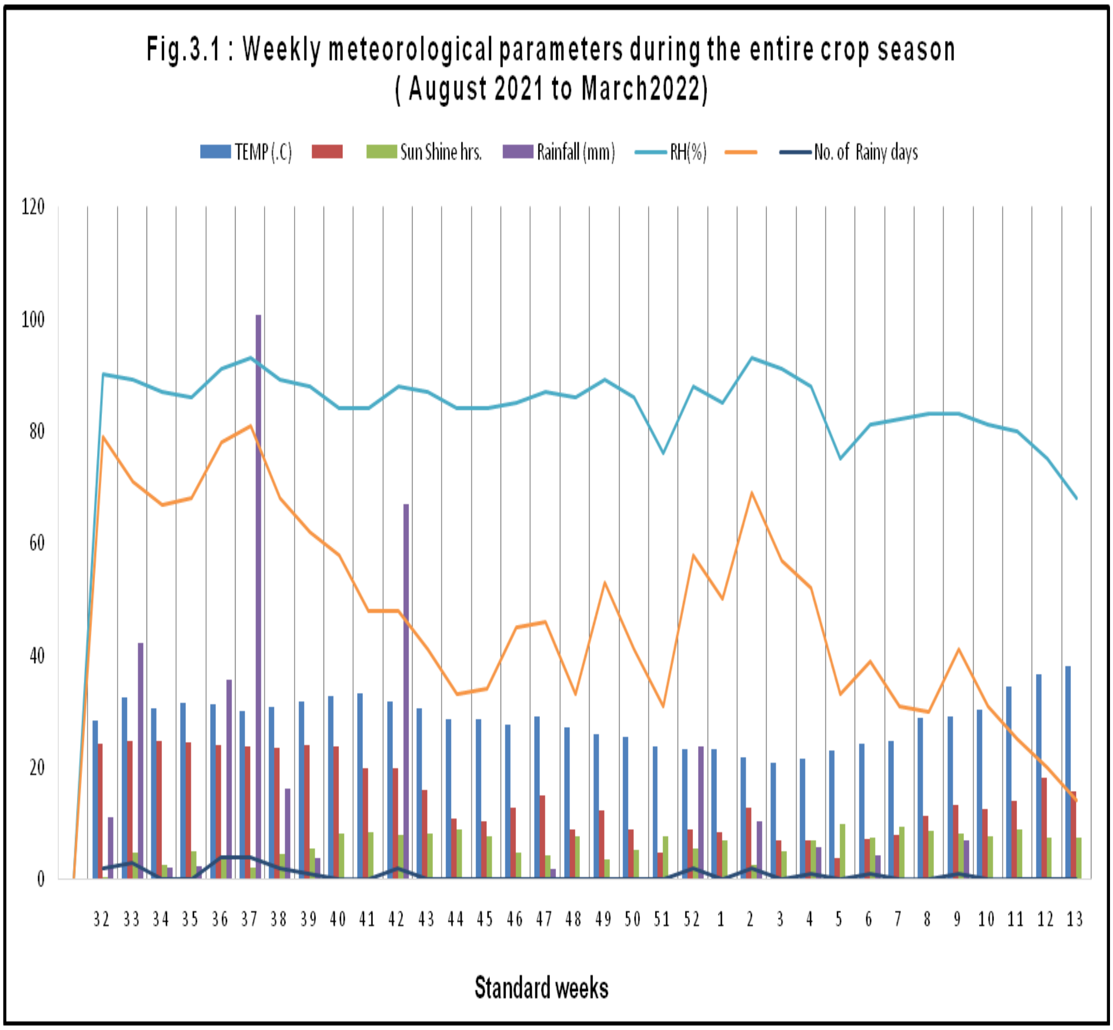


Fig.3.1 : Weekly meteorological parameters during the entire crop season
(August 2021 to March2022)
TEMP (.C)
Sun Shine hrs.
Rainfall (mm)
RH(%)
No. of Rainy days
120
100
80
60
40
20
0
32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 1 2 3 4 5 6 7 8 9 10 11 12 13
Standard weeks

3.3 Soil Characteristics of the experimental site:

The soil of this region was Vertisol or deep black soil with sandy clay-loam texture. These clayey soils shrink and swell extensively upon changing soil moisture conditions.

3.4 Experimental details

The experimental layout details were given below in the Table as follows:

Table3.2: Details of the experiment

Location	Experimental field of AICRP on Medicinal and Aromatic Plants JNKVV , Jabalpur
Crop	Ashwagandha
Variety	JA 20
Season	*Late Kharif* (2021-2022)
Experimental Design	Randomized Block Design
Treatment	7
Replications	3
Number of plots	21
Distance between rows	30cm
Distance between plots	0.5m
Distance between replications	1m
Gross plot size	24.5 m x 9 m [220.5 m^2]
Net plot size	21 m x 6.5 m[136.5 m^2]
Date of sowing	2Sept' 2021
Date of harvesting	11 march' 2022

Table3.3: Different treatments with different dose

T. No.	Treatment details
T_1	Control (untreated)
T_2	Nanocombi (Zn+Mn+Cu) @ 10mgL^{-1}
T_3	Nanocombi (Zn+Mn+Cu) @ 20mgL^{-1}
T_4	Nanocombi (Zn+Mn+Cu) @ 30mgL^{-1}
T_5	Nanocombi (Zn+Mn+Cu) @ 40mgL^{-1}
T_6	Nanocombi (Zn+Mn+Cu) @ 50mgL^{-1}
T_7	Nanocombi (Zn+Mn+Cu) @ 60mgL^{-1}

3.5 Experimental materials

The Ashwagandha (*Withania somnifera* (L.) Dunal) cv Jawahar Aswagandh 20 was sown in each plot. Nano micronutrients were applied in the form of Nanocombi (Zn+Mn+Cu) of Geolife company with (Zn 16.6%, Mn 3.8% and Cu 3.8%) of different concentrations. Well Decomposed FYM was applied in each plot 10-15 days before sowing.

3.6 Experimental Design and Layout

The description of design and layout is as follows:

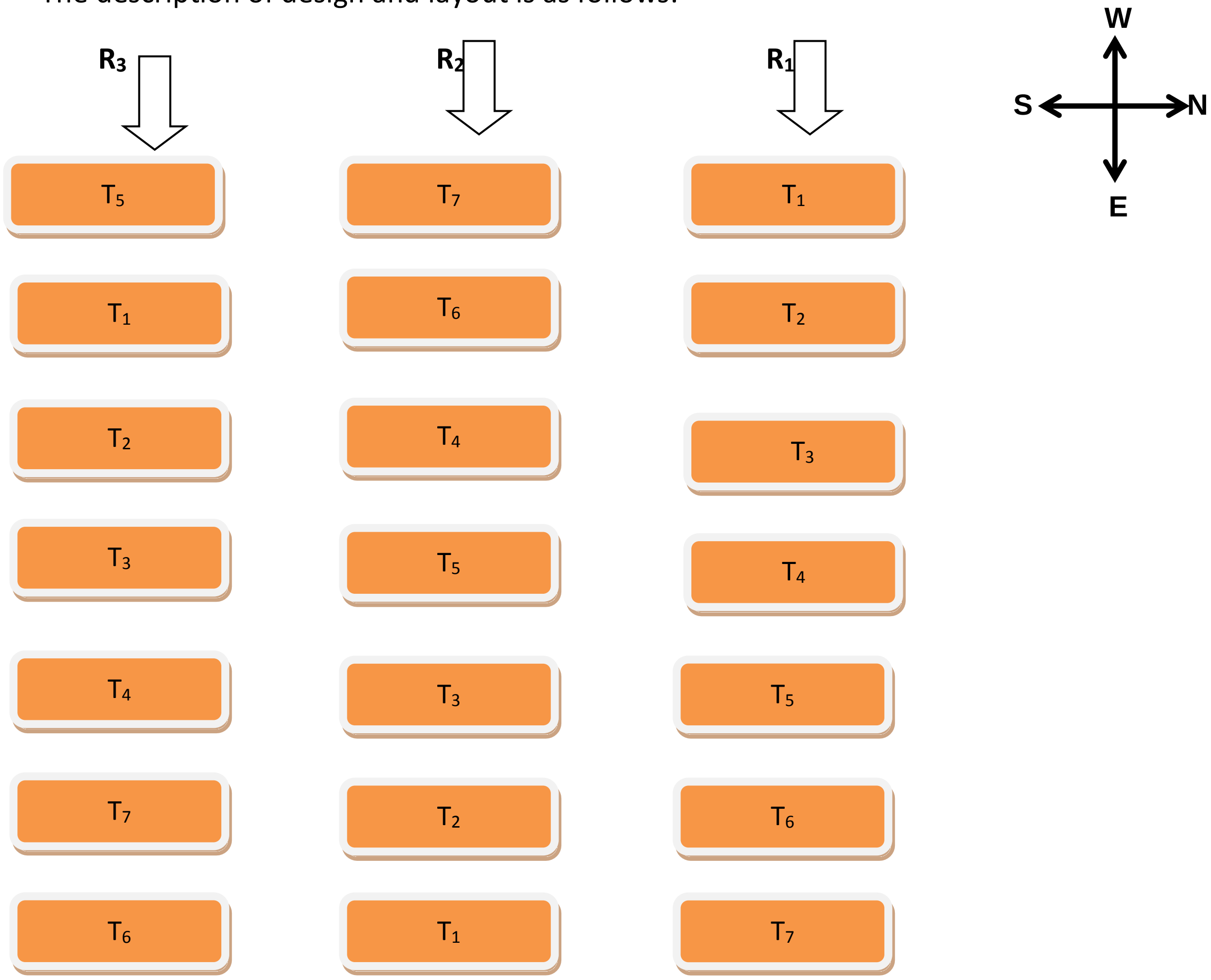


Fig 3.2: Layout Plan of Experimental Design

3.7 Cultural Operation

3.7.1 Pre sowing operation:

The field was ploughed uniformly with the help of cultivator. Thereafter field was leveled with heavy wooden plank and plots of equal size were made as per the layout plan.

3.7.2 Seed sowing

The seeds of Ashwagandha (JA 20) were sown at the rate of 7.5gm plot^{-1} by hand at depth of 3-4cm in open furrow.

3.7.3 Thinning

Thinning was done manually after 15 days of germination to maintain the uniform and desired plant population.

3.7.4 Application of nutrients (fertilizer)

As Ashwagandha is one of the medicinal crop. Therefore chemical fertilizer is strictly prohibited so as to maintain the quality of the produce. Hence, Ashwagandha is mostly grown organically by applying well decomposed FYM at time of field preparation. In this research work we used different concentration of Nano Combi (Zn+Cu+Mn) so as to fulfill the micronutrient requirement of the crop without detoriating the quality of produce. This Nanocombi is given as a foliar spray at 30 days interval. Two sprays was sufficient one given at 30 DAS and second at 30 days after 1st spray.

3.7.5 Intercultural operation

Two times weeding was done first at 30DAS by manual laborers and second weeding done at 45DAS so as to minimize crop weed competition for light, space, nutrient and moisture.

3.7.6 Harvesting

The crop was ready to harvest in 180-190 days which was commenced in March. Maturity was judged by drying of leaves and yellowish-red colour of berries. The whole plant was uprooted at time of harvest. Aerial part was separated by cutting the stem 1-2cm above the crown and roots were collected.

3.8 Crop Studies Conducted:

3.8.1 Sampling : Sampling was done at definite interval for growth and yield attributes viz plant height , number of branches and number of leaves by randomly taking five plants at 30,60,90 DAS respectively and at harvesting.

3.9 Observation to be taken:

3.9.1 Phenological observation: The phenological observations were recorded by taking five plants from each plot throughout the growth period. Following were the growth stage which has been observed:

Days to seedling emergence

Days to initiation of primary branching

Days to initiation of secondary branching

Days to Floral initiation

Days to 50%flowering

Days to Fruit initiation

Days to 50% fruiting

Days to seed formation

Days to 1st fruit maturity

Days to 50% fruit maturity

3.9.2 Physiological observation :

Number of leavesplant^{-1}

Leaf area Index (Gardner *et al.,* 1985)

LAI expresses the ratio of leaf surface (one side only) to the ground area occupied by the plant or a crop stand worked out as per specifications of Gardner *et al*. (1985).

$$\textbf{LAI} = \frac{\text{Total leaf area}}{\text{Land area}} \qquad \textbf{LAI} = \frac{(LA_2 + LA_1)}{2xp}$$

Where,

LA1 and LA2 represent leaf area during two consecutive intervals and ‘P’ ground area.

Leaf Area Duration (cm^2 days) (Watson, 1982)

Leaf area duration expresses the magnitude and persistence of leaf area or leafiness during the period of crop growth. It reflects the extent of seasonal integral of light interaction and correlated with yield. LAD was computed as follows (Watson, 1952).

$$\textbf{LAD} = \frac{(LA_2 + LA_1)}{2} \times (t_2 - t_1)\ (cm^2.days)$$

Where,

LA_1 and LA_2 represent the leaf area at two successive time intervals (t_1 and t_2).

3.9.3 Biochemical Parameters:

Chlorophyll content Index (SPAD-502)

The SPAD 502 plus chlorophyll meter instantly measures chlorophyll content or “greenness” of plants. Simply clamp the meter over leafy tissue, and receive on indexed chlorophyll content reading (-9.9 to 199.9) in less than 2 seconds.

Proline content

Proline content at different growth stages had been calculated with the help of Bates method.

3.9.4 Yield & yield attributes:

Plant height (cm)

The height of the plants from five tagged plants was measured from base to the top most leaf. The observations were recorded at 30, 60, 90, 120 DAS and at harvest. Then the average was computed.

No: of branches $plant^{-1}$

The primary and secondary branches were counted in all five tagged plants at 30, 60, 90, 120 DAS and at harvest. Then the mean was worked out.

Leaf biomass (g)

The leaf biomass was calculated from all five tagged plants at 30, 60, 90, 120 DAS and at harvest. Then the mean was worked out.

Number of seeds pod^{-1} $plant^{-1}$

The number of seeds pod^{-1} recorded from five tagged plants at maturity in each plot and the mean was calculated

Root weight plant^{-1} (g plant^{-1})

Root length, root diameter dry root weight from all five tagged plants had been measured at time of harvest.

Leaf yield (kgha^{-1})

Leaf yield kgha^{-1} was recorded after drying the leaves.

Root yield (kgha^{-1})

Root yield kgha^{-1} was recorded after cleaning and drying the roots. It is known as economical part of the crop and has multiple medicinal uses.

Seed yield (kgha^{-1})

The seed yield kgha^{-1} was recorded after threshing, cleaning and drying the seeds. It is also known as economical part.

Harvest Index (%)

Harvest index is the ratio of economic yield to the total biological yield expressed in percentage. It represents the efficiency of photosynthesis translocation to economic parts (Synder and Carlson, 1984).

$$HI(\%) = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

3.9.5 Active ingredient analysis :

Withanolide A

3.10 Yield Parameters

Post harvest data such as length of roots, thickness of root, dry root weight plot^{-1} and fresh root weight plot^{-1} were recorded from randomly selected plots. The plants from each plot were selected, uprooted, roots were separated and oven dried for 3-4 days.

The data collected was statistically analyzed using ANNOVA procedures.

3.11 Quality parameters

Plants were harvested and separated into roots, leaves, stem immediately. Oven dried the samples and then Quantitative Analysis for Proline content and total Withanolide content was done separately for each treatments using method given below:

3.11.1 Proline estimation:

Proline content was estimated by Bates method.

Reagents:

1. Sulphosallysic acid (3%): Three gram of sulphosalycylic acid was dissolved in 100 ml of distilled water
2. Orthophosphoric acid (6N): Required volume of orthophosphoric acid (38.1 ml) was taken and volume was made to 100 ml, using distilled water to get 6 N orthophosphoric acid.
3. Acid Ninhydrin: Ninhydrin (1.25 g) was dissolved in a blend of 30 ml of glacial acetic acid and 20 ml of 6 N orthophosphoric acids with agitation until dissolved store at 4°C and use within 24 hours.
4. Glacial acetic acid.
5. Toluene
6. Proline 50 mg in 50 ml D.W.

Procedure:

1. Extract 0.5 g of plant material by homogenizing in 10 ml of 3% aqueous sulphosalicylic acid.
2. Filter the homogenate through Whatman No.2 filter paper.
3. Take 2ml of filtrate in a test tube a test tube and 2 ml of glacial acetic acid and 2 ml acid-ninhydrin.
4. Heat it in boiling water bath for 1 hour.
5. Terminate the reaction by placing the tube in ice bath.
6. Add 4 ml toluene to the reaction mixture and stir for 20-30sec.
7. Separate the toluene layer and warm to room temperature.
8. Measure the red colour intensity at 520 nm.

9. Run a series of standard with pure proline in a similar way and prepare a standard curve.

10. Find out the amount of proline in the test sample from the standard curve.

Calculation:

Express the proline content on fresh-weight-basis as follows:

$$\text{mmoles per g tissue} = \frac{\text{mg proline/mL} \times \text{mL toluene}}{115.5} \times \frac{5}{\text{g sample}}$$

where 115.5 is the molecular weight of proline.

3.11.2 Total Withanolide estimation:

Withanolide content in ashwagandha crop was estimated by HPTLC method.

Sample Preparation:

1. 2gm of each finely powdered sample was extracted three times with 3.0ml methanol by sonication for 10 minute.
2. Centrifuged the extract for 5 minute at 3000 rev/min.
3. Reduced under vacuum.
4. The extracts were combined in a 10ml volumetric flask and adjusted to the final volume with methanol.
5. Now sample were diluted with 1:1 methanol.
6. Prior to use, all the samples were filtered through a 0.45µm filter paper.

Analytical method by HPTLC

1. Mobile Phase: Toluene: Ethyl acetate: Formic acid (5:5:1)

2. Detection: 200nm

3. Procedure: Apply 10µl of the reference and sample solutions on the different tracks on the silica gel plate (10cm 10cm) of uniform thickness

(0.2mm thickness). Develop the plate in the solvent system up to a distance of 8cm.

4. Scanning: Scanning the plate using a Camag TLC Scanner at 200nm for both reference and test solution tracks. Peak purity tests were carried out by comparing their peak areas and RF values of withanolide A & B and withaferin A (0.24, 0.33 and 0.46) respectively with those present in the reference and test solution tracks.

5. Visualization of spots (Post scanning): Freshly prepared p anisaldehyde reagent is used. After drying, the plate was heated at 110°C for 10 min to develop the color of the spots.

Statistical analysis:

Analysis of observations taken on different variables was carried out to know the degree of variation among all the treatments. The pooled data was statistically analyzed through randomized block design (Fisher, 1967). The results obtained through analysis of variance are given in appendix and the skeleton of analysis of variance table is given below:

Table 3.4 Analysis of variance (ANOVA)

S. No.	Sources of variance (S.V.)	Degree of freedom (d.f.)	Sum of squares (S.S.)	Mean sum of squares (MSS)	Calculated d.f. value	Table value 5% 1%
1.	Replications	(r-1)				
2.	Treatments	(t-1)				
3.	Error	(r-1)(t-1)				
4.	Total	rt-1				

S.E. (m) ± = EMS/2

S.E. (d) ± = 2EMS/r

C.D. = S.E. (d) X t5% at error d.f.

Where,

R	= number of replications
T	= number of treatments
E.M.S	= Error mean square
S.E. (m)	= Standard error of treatment means
S.E. (d)	= Standard error of difference to two treatment mean
C.D.	= Critical difference of two treatment means

RESULTS

The present research work was carried out to assess the effect of Nano-micronutrients (Zn + Mn + Cu) on phenology, physiology, growth, productivity and quality parameters under the experiment entitled **"Optimization of Nano-micronutrients for phenology, growth, productivity and quality of *Withania somnifera* (L.) Dunal."** The observed data taken at different growth stages analyzed statistically and the experimental results of the present research work have been presented under following subheads:

4.1 Phenological parameters

4.1.1 Days to seedling emergence

4.1.2 Days to initiation of primary branching

4.1.3 Days to initiation of secondary branching

4.1.4 Days to Floral initiation

4.1.5 Days to 50%flowering

4.1.6 Days to Fruit initiation

4.1.7 Days to 50% fruiting

4.1.8 Days to seed formation

4.1.9 Days to 1^{st} fruit maturity

4.1.10 Days to 50% fruit maturity

4.2 Physiological parameters

4.2.1 Number of leaves plant^{-1}

4.2.2 Leaf area Index(Gardner *et al.,* 1985)

4.2.3 Leaf Area Duration (cm^2 days) [Watson, 1982]

4.3 Biochemical Parameters

4.3.1 Chlorophyll content Index (SPAD-502)

4.3.2 Proline content

4.4 Yield & yield attributes

4.4.1 Plant height (cm)

4.4.2 No: of branches plant^{-1}

4.4.3 Leaf biomass ($kgha^{-1}$)

4.4.4 Number of seeds pod^{-1} $plant^{-1}$

4.4.5 Root Parameters(root length, root diameter, root dry weight)

4.4.6 Yield (leaf, root and seed yield)

4.4.7 Harvest Index (%)

4.5 Active ingredient analysis

4.5.1 Withanolide A

4.1 Phenological parameters

Phenological characters *viz*., days to seedling emergence, days to primary branching , days to secondary branching , days to floral initiation, days to 50% flowering, days to fruit initiation, days to 50% fruiting, days to seed formation, days to 1st fruit maturity and days to 50% maturity were studied in Ashwagandha under the influence of treatments are presented in Table 4.1 to 4.2 and illustrated as Fig.4.1 to 4.2.

4.1.1 Days to seedling emergence

The results revealed that treatment T_1 (12.67) required maximum days to seedling emergence at par with T_6 and T_7 (12.33) followed by T_2 (11.33), T_4 (11.33) and T_3 (10.67). Minimum days required for seedling emergence was observed in T_5 (10.33).

4.1.2 Days to primary branching

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum days required to initiate primary branching was recorded in treatment T_1 (43.67) which was at par with T_7 (43.33), T_2 (43.00) and T_6 (42.33), followed by T_3 (41.67) and T_5 (41.00). Minimum days required for primary branching was observed in T_4 (40.67).

4.1.3 Days to secondary branching

Nano micronutrients (Zn + Mn + Cu) spray noted significant variation in days required to initiate secondary branching ,as maximum days required was recorded in treatment T_1 (54.33) which was at par with T_7 (54.00), T_2

(53.00) and T_6 (53.00), followed by T_3 (52.33) and T_5 (51.67). Minimum days required for secondary branching was noted in T_4 (51.33).

4.1.4 Days to floral initiation

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) on Ashwagandha crop (*Withania somnifera* (L.) Dunal) showed significant effect as maximum days required to initiate flowering was recorded in treatment T_1 (68.33) and T_7 (68.33) which was at par with T_2 (67.33), T_5 (67.00) and T_6 (67.00), followed by T_4 (66.67). Minimum days required for initiating flowering was seen in treatment T_3 (65.33).

4.1.5 Days to 50% flowering

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) on Ashwagandha crop (*Withania somnifera* (L.) Dunal) showed significant effect as maximum days required to reach 50% flowering was recorded in treatment T_1 (79.67), which was at par with T_7 (79.33) and T_2 (78.67), followed by T_6 (77.33),T_5 (76.33) and T_4 (75.67).While minimum days required for reaching 50% flowering was seen in treatment T_3 (75.33).

Table 4.1: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu)on phenological parameters of ashwagandha crop

Treatment	Days to seedling emergence	Days to primary branching	Days to secondary branching	Days to floral initiation	Days to 50% flowering
T_1 Control (untreated)	12.67	43.67	54.33	68.33	79.67
T_2 Nanocombi @ 10mgL^{-1}	11.33	43.00	53.00	67.33	78.67
T_3 Nanocombi @ 20mgL^{-1}	10.67	41.67	52.33	65.33	75.33
T_4 Nanocombi @ 30mgL^{-1}	11.33	40.67	51.33	66.67	75.67
T_5 Nanocombi @ 40mgL^{-1}	10.33	41.00	51.67	67.00	76.33
T_6 Nanocombi @ 50mgL^{-1}	12.33	42.33	53.00	67.00	77.33
T_7 Nanocombi @ 60mgL^{-1}	12.33	43.33	54.00	68.33	79.33
S.Em±	0.3600	0.5516	0.5842	0.5394	0.3563
CD(0.05)	1.1094	1.6996	1.8000	1.6622	1.0980

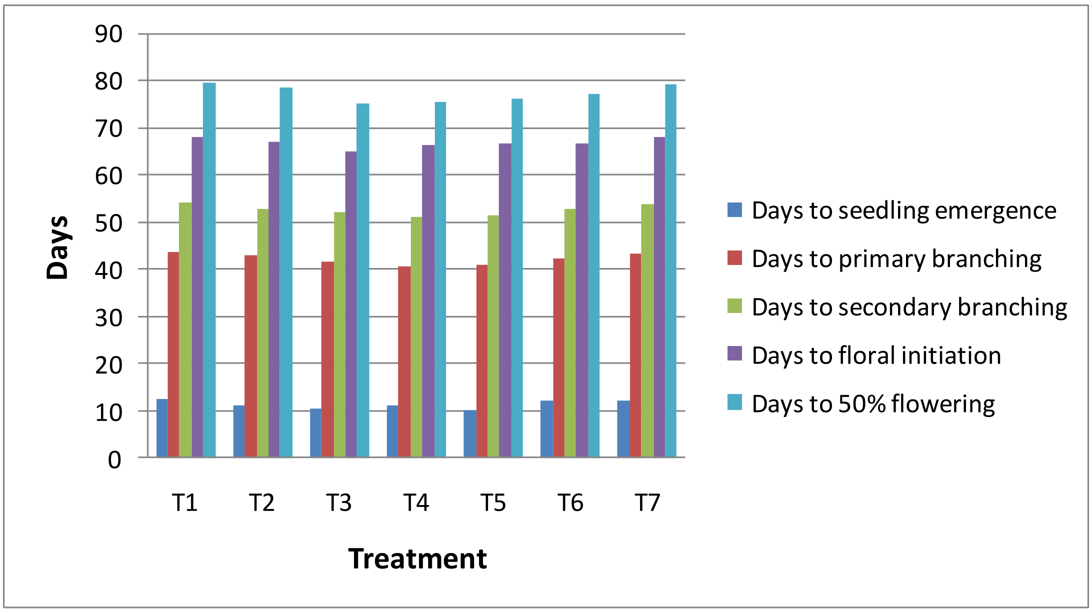


Fig.4.1: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu)on phenological parameters of ashwagandha crop

4.1.6 Days to fruit initiation

Nano micronutrients (Zn + Mn + Cu) spray noted significant variation in days required to initiate fruiting as maximum days required to initiate fruiting was recorded in treatment T_6 (102.33) which was at par with T_1 (102.00), followed by T_2 (98.33), T_7 (97.67), T_3 (96.67) and T_5 (96.33). Minimum days required for initiating fruiting was seen in treatment T_4 (95.67).

4.1.7 Days to 50% fruiting

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) on Ashwagandha crop (*Withania somnifera* (L.) Dunal) showed significant effect as maximum days required to attain 50% fruiting was recorded in treatment T_1 (119.67), which was followed by T_6 (118.33), T_2 (117.67), T_3 (117.33), T_7 (117.33) and T_5 (116.67). Minimum days required for attaining 50% fruiting was seen in treatment T_4 (115.33).

4.1.8 Days to seed formation

Nano micronutrients (Zn + Mn + Cu) spray noted significant variation in days required to seed formation, as maximum days required to seed formation was noted in treatment T_1 (134.33), which was at par with T_6

(133.33) and T_7 (133.33), followed by T_5 (132.67), T_2 (132.33) and T_4 (131.67). Minimum days required for seed formation was seen in treatment T_3 (130.67).

4.1.9 Days to 1st fruit maturity

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal) as maximum days required for fruit maturity was observed in treatment T_1 (149.33), which was followed by T_5 (147.67), which was at par with T_2, T_6 and T_7 (147.33) followed by T_4 (146.00). Minimum days required for fruit maturity was seen in treatment T_3 (145.67).

4.1.10 Days to 50% maturity

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) on Ashwagandha crop (*Withania somnifera* (L.) Dunal) showed significant effect as maximum days required to attain 50% maturity was recorded in treatment T_1 (169.33), which was at par with T_2 (167.67), followed by T_5 (167.33), T_6 (167.00), T_7 (167.00) and T_4 (166.67). Minimum days required for attaining 50% maturity was seen in treatment T_3 (165.33).

Table 4.2: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu)on phenological parameters of ashwagandha crop

Treatment	Days to fruit initiation	Days to 50% fruiting	Days to seed formation	Days to 1st fruit maturity	Days to 50% maturity
T_1 Control (untreated)	102.00	119.67	134.33	149.33	169.33
T_2 Nanocombi @ $10mgL^{-1}$	98.33	117.67	132.33	147.33	167.67
T_3 Nanocombi @ $20mgL^{-1}$	96.67	117.33	130.67	145.67	165.33
T_4 Nanocombi @ $30mgL^{-1}$	95.67	115.33	131.67	146.00	166.67
T_5 Nanocombi @ $40mgL^{-1}$	96.33	116.67	132.67	147.67	167.33
T_6 Nanocombi @ $50mgL^{-1}$	102.33	118.33	133.33	147.33	167.00
T_7 Nanocombi @ $60mgL^{-1}$	97.67	117.33	133.33	147.33	167.00
S.Em±	0.4629	0.4272	0.5270	0.5245	0.5587
CD(0.05)	1.4264	1.3165	1.6240	1.6162	1.7216

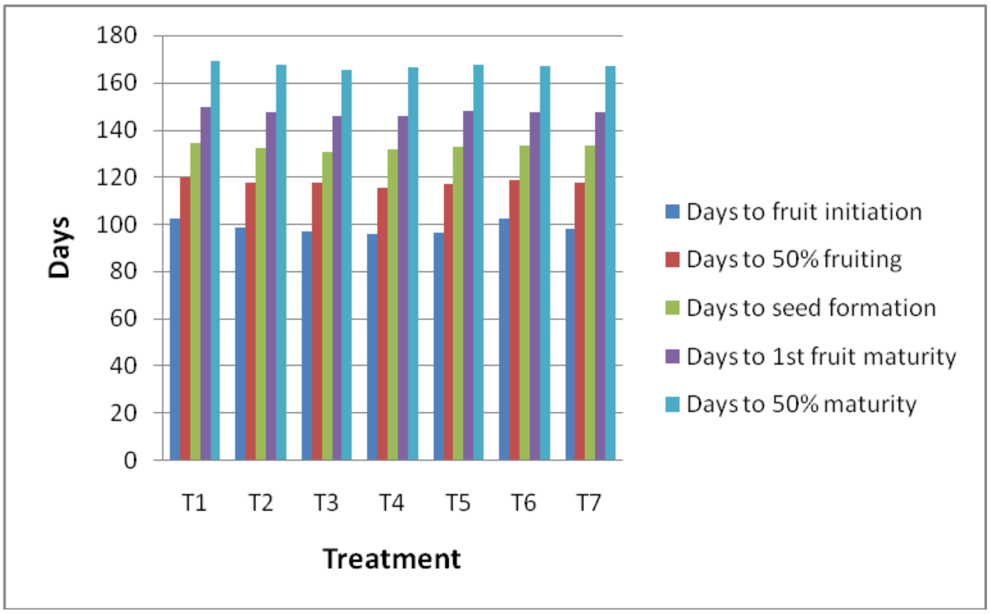


Fig.4.2: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on phenological parameters of ashwagandha crop

4.2 Physiological Parameters

4.2.1 Number of Leaves per plant

Number of leaves per plant was significantly affected by spraying Nano- micronutrients (Zn + Mn + Cu) after 1st spray at 30 DAS till maturity which was presented in Table 4.3 and illustrated in Fig.4.3

At 30 DAS

The results showed that maximum number of leaves were obtained in T_3 (6.73) which was at par with T_4 (5.87), followed by T_6 (5.33), T_5 (5.20), T_7 (4.87) and T_2 (4.33), while minimum leaves were seen in T_1 (4.00).

At 60 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum number of leaves per plant was recorded in treatment T_5 (43.40) which was at par with T_4 (42.67), and T_3 (42.50), followed by T_6 (37.87), T_7 (37.47) and T_2 (36.00), while minimum number of leaves was observed in T_1 (35.13).

At 90 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum number of leaves per plant was recorded in treatment T_5 (63.00) which was followed by T_4 (57.67), T_6 (57.10), T_7 (56.70), T_3 (53.00) and T_1 (50.47), while minimum number of leaves was noted in T_2 (50.43).

At 120 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) on Ashwagandha crop (*Withania somnifera* (L.) Dunal) showed significant effect as maximum number of leaves per plant was recorded in treatment T_5 (74.00) which was at par with T_4 (72.80), followed by T_6 (65.67), T_7 (65.07), T_3 (64.37) and T_1 (63.40), while minimum number of leaves was observed in T_2 (62.93).

At Harvest

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum number of leaves per plant was noted in treatment T_5 (33.67) which was at par with T_4 (32.10), which was followed by T_3 (30.53), T_7 (26.83), T_6 (26.73), T_2 (26.73), while minimum number of leaves was noted in T_1 (24.17).

Table.4.3: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on number of leaves plant^{-1} at different stages of the crop

Treatment	30DAS	60DAS	90DAS	120DAS	At Harvest	Mean
T_1 Control (untreated)	4.00	35.13	50.47	63.40	24.17	35.43
T_2 Nanocombi @ 10mgL^{-1}	4.33	36.00	50.43	62.93	26.73	36.08
T_3 Nanocombi @ 20mgL^{-1}	6.73	42.50	53.00	64.37	30.53	39.42
T_4 Nanocombi @ 30mgL^{-1}	5.87	42.67	57.67	72.80	32.10	42.22
T_5 Nanocombi @ 40mgL^{-1}	5.20	43.40	63.00	74.00	33.67	43.85
T_6 Nanocombi @ 50mgL^{-1}	5.33	37.87	57.10	65.67	26.73	38.54
T_7 Nanocombi @ 60mgL^{-1}	4.87	37.47	56.70	65.07	26.83	38.18
S.Em±	0.3429	0.8206	0.7656	0.4837	0.7926	
CD(0.05)	1.0565	2.5284	2.3591	1.4904	2.4421	

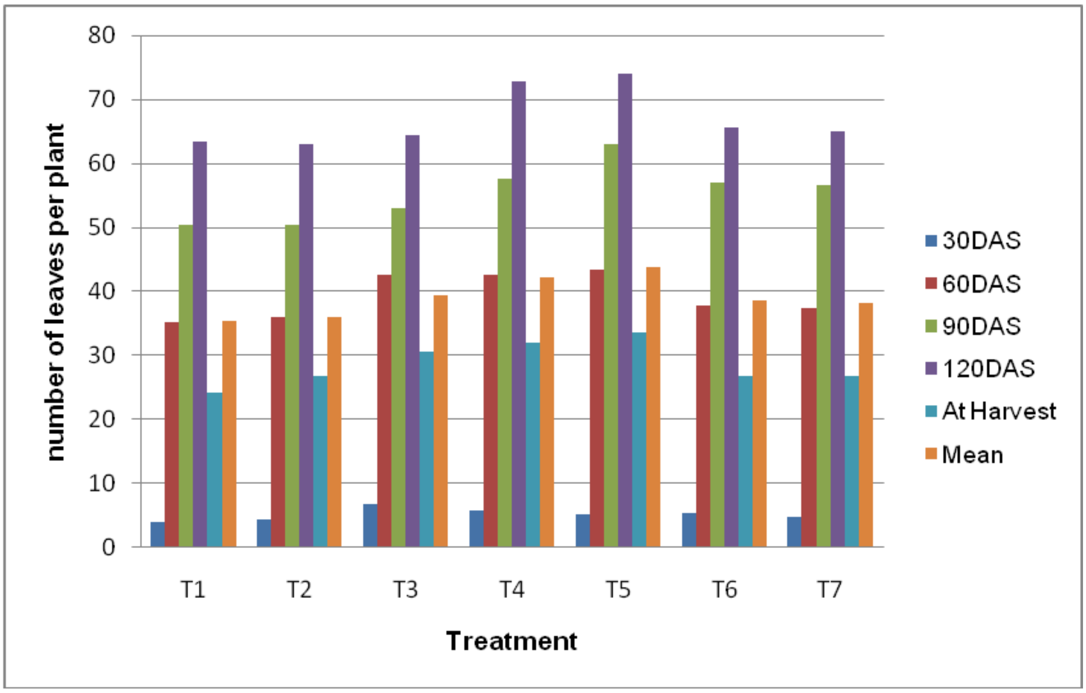


Fig.4.3: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on number of leaves plant^{-1} at different stages of the crop

4.2.2 Leaf Area Index (LAI)

Leaf Area Index was significantly affected by spraying Nano-micronutrients (Zn + Mn + Cu) after 60DAS till maturity which was presented in Table 4.4 and illustrated in Fig.4.4.

At 30 DAS

The results revealed non-significant effect that maximum LAI was recorded in T_3 (0.17) which was at par with all the other treatments T_4 (0.15), followed by T_6 (0.14), T_7 (0.13), T_5 (0.12),T_2 (0.12) and T_1 (0.12).

At 60 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed non-significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum LAI was recorded in treatment T_5 (2.66) which was at par with all the other treatments T_3 (2.61), T_4 (2.59), T_6 (2.47), T_2 (2.45) T_7 (2.40), and T_1 (2.32).

At 90 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum LAI was noted in treatment T_4 (3.34) which was at par with T_5 (3.25), which was followed by T_3 (2.81), T_1 (2.37), T_2 (2.37), T_7 (2.37), while minimum LAI was noted in T_6 (2.36).

At 120 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) on Ashwagandha crop (*Withania somnifera* (L.) Dunal) showed significant effect, as maximum LAI was observed in treatment T_4 (2.42) which was at par with T_5 (2.39) and T_3 (2.26), which was followed by T_2 (2.04), T_7 (1.95), T_6 (1.91), while minimum LAI was recorded in T_1 (1.84).

At Harvest

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) on Ashwagandha crop (*Withania somnifera* (L.) Dunal) showed significant effect, as maximum LAI was recorded in treatment T_4 (1.77) which was followed by T_5 (1.56), T_3 (1.42), T_6 (1.35), T_2 (1.32), T_1 (1.25), while minimum LAI was recorded in T_7 (1.23).

Table.4.4: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on LAI at different stages of the crop

Treatment	30DAS	60DAS	90DAS	120DAS	At Harvest	Mean
T_1 Control (untreated)	0.12	2.32	2.37	1.84	1.25	1.58
T_2Nanocombi @ 10mgL^{-1}	0.12	2.45	2.37	2.04	1.32	1.66
T_3 Nanocombi @ 20mgL^{-1}	0.17	2.61	2.81	2.26	1.42	1.85
T_4 Nanocombi @ 30mgL^{-1}	0.15	2.59	3.34	2.42	1.77	2.05
T_5 Nanocombi @ 40mgL^{-1}	0.12	2.66	3.25	2.39	1.56	1.99
T_6 Nanocombi @ 50mgL^{-1}	0.14	2.47	2.36	1.91	1.35	1.64
T_7 Nanocombi @ 60mgL^{-1}	0.13	2.40	2.37	1.95	1.23	1.61
S.Em±	0.0173	0.1164	0.1248	0.1027	0.0541	
CD(0.05)	0.0532	0.3587	0.3846	0.33	0.1667	

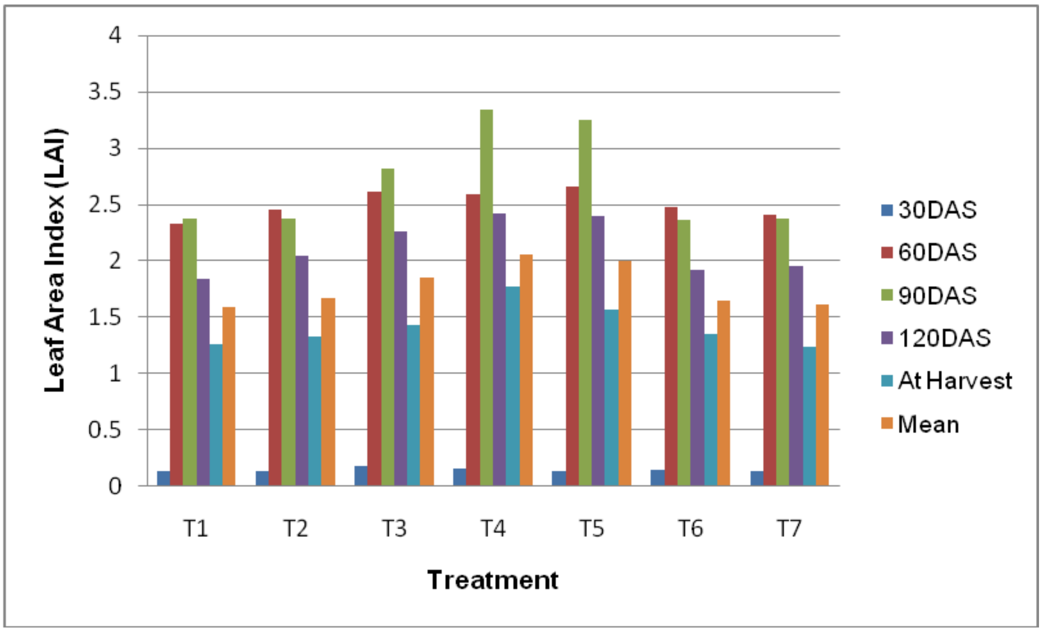


Fig.4.4: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on LAI at different stages of the crop

4.2.3 Leaf Area Duration (LAD)

The data regarding the LAD of Ashwagandha crop are presentedin Table 4.5 and Fig. 4.5. Leaf Area Duration of ashwagandha recorded at 30-60, 60-90, 90-120 DAS and 120- harvest which was significantly affected by foliar application of nano micronutrients at 30 and 60 DAS.

At 30-60 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum LAD was recorded in treatment T_3 (40.51) which was at par with T_5 (38.56), and T_7 (37.24), followed by T_6 (35.96), T_4 (35.66) and T_2 (33.97), while minimum LAD was observed in T_1 (31.32).

At 60-90 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum LAD was noted in treatment T_3 (85.97) which was at par with T_4 (83.66), followed by T_5 (81.43), T_7 (76.43) ,T_2 (74.92) and T_1 (74.64),, while minimum LAD was observed in T_6 (74.18).

At 60-120 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum LAD was observed in treatment T_4 (72.66) which was at par with T_5 (71.44), and T_3 (69.26), followed by T_6 (68.42), T_2 (66.88) and T_1 (66.84), while minimum LAD was observed in T_7 (65.54).

At 120 DAS-Harvest

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum LAD was observed in treatment T_5 (69.00) which was followed by T_4 (61.63), T_3 (52.39), T_7 (51.90), T_6 (48.05) and T_2 (46.31), while minimum LAD was recorded in T_1 (44.04).

Table.4.5: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on LAD at different stages of the crop

Treatment	30-60DAS	60-90DAS	90-120 DAS	120-At Harvest	Mean
T_1 Control (untreated)	31.32	74.64	66.84	44.04	54.21
T_2 Nanocombi @ 10mgL^{-1}	33.97	74.92	66.88	46.31	55.52
T_3 Nanocombi @ 20mgL^{-1}	40.51	85.97	69.26	52.39	62.03
T_4 Nanocombi @ 30mgL^{-1}	35.66	83.66	72.66	61.63	63.40
T_5 Nanocombi @ 40mgL^{-1}	38.56	81.43	71.44	69.00	65.10
T_6 Nanocombi @ 50mgL^{-1}	35.96	74.18	68.42	48.05	56.65
T_7 Nanocombi @ 60mgL^{-1}	37.24	76.43	65.54	51.90	57.77
S.Em±	1.1202	0.6993	1.1290	1.6912	
CD(0.05)	3.4517	2.1549	3.4789	5.2111	

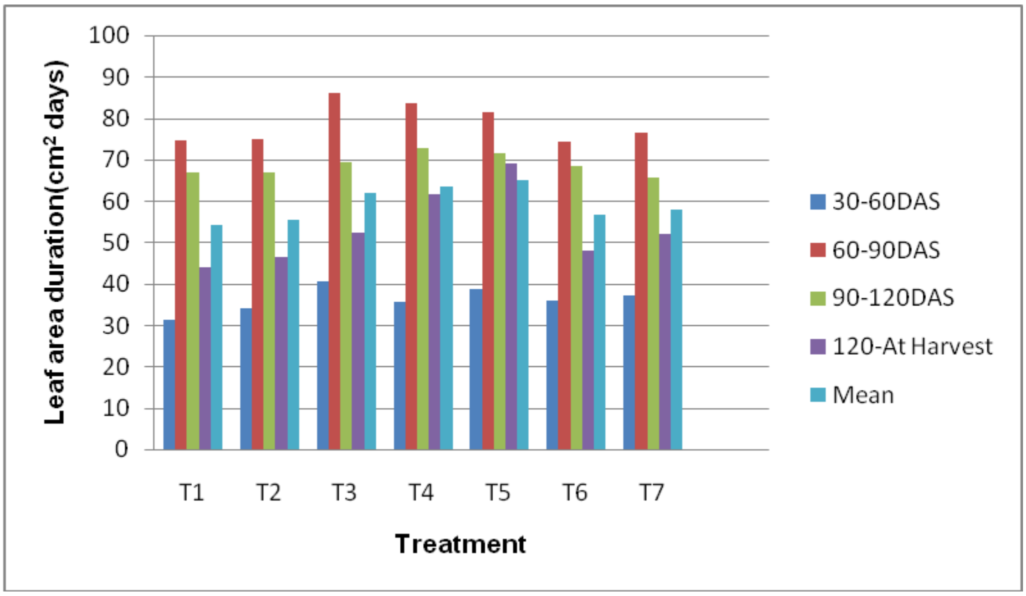


Fig.4.5: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on LAD at different stages of the crop

4.3 Biochemical Parameters

4.3.1 Chlorophyll Content Index

Data regarding chlorophyll content in leaves recorded at different growth stages are given in Table 4.6 and illustrated as Fig 4.6. The chlorophyll content in leaves recorded at different growth stages was significantly influenced by foliar spray of nano-micronutrients on Ashwagandha crop.

At 30 DAS

The results showed that maximum chlorophyll content were obtained in T_4 (63.48) which was at par with T_5 (62.43) and T_6 (60.21), followed by T_3 (58.96), T_7 (57.91) and T_2 (54.33), while minimum chlorophyll content seen in T_1 (51.27).

At 60 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum chlorophyll content was recorded in treatment T_4 (76.44) which was followed by T_5 (73.33), T_6 (71.93), T_7 (67.99), T_3 (66.80) and T_2 (60.24), while minimum chlorophyll content was observed in T_1 (57.18).

At 90 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum chlorophyll content was recorded in treatment T_3 (82.11) which was followed by T_4 (80.31), T_5 (79.62), T_7 (76.55), T_6 (75.08) and T_2 (72.43), while minimum chlorophyll content was noted in T_1 (69.51).

At 120 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum chlorophyll content was observed in treatment T_4 (44.76) which was at par with T_5 (43.57) and T_5 (42.97), followed by T_3 (41.60), T_7 (41.55) and T_2 (36.45), while minimum chlorophyll content was noted in T_1 (35.97).

At Harvest

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum chlorophyll content was observed in treatment T_4 (19.74) which was followed by T_5 (18.06), T_3 (16.78), T_7 (16.25), T_6 (15.76) and T_2 (13.07), while minimum chlorophyll content was recorded in T_1 (11.81).

Table.4.6: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Chlorophyll Content Index at different stages of the crop

Treatment	30DAS	60DAS	90DAS	120DAS	At Harvest	Mean
T_1 Control (untreated)	51.27	57.18	69.51	35.97	11.81	45.14
T_2Nanocombi @ 10mgL^{-1}	54.33	60.24	72.43	36.45	13.07	47.30
T_3 Nanocombi @ 20mgL^{-1}	58.96	66.80	82.11	41.60	16.78	53.25
T_4 Nanocombi @ 30mgL^{-1}	63.48	76.44	80.31	44.76	19.74	56.94
T_5 Nanocombi @ 40mgL^{-1}	62.43	73.33	79.62	43.57	18.06	55.40
T_6 Nanocombi @ 50mgL^{-1}	60.21	71.93	75.08	42.97	15.76	53.19
T_7 Nanocombi @ 60mgL^{-1}	57.91	67.99	76.55	41.55	16.25	52.05
S.Em±	1.2060	0.7562	0.5101	0.6711	0.3721	
CD(0.05)	3.7161	2.3300	1.5719	2.0678	1.1467	

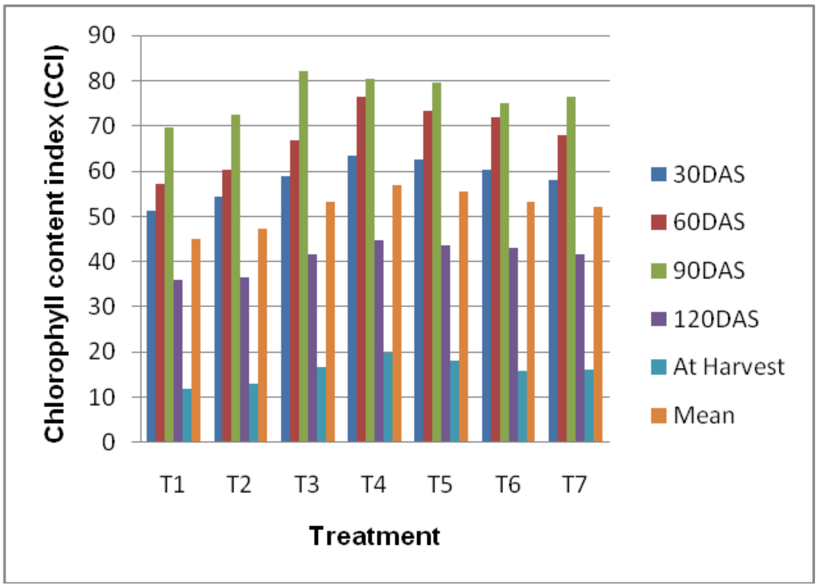


Fig.4.6: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Chlorophyll Content Index at different stages of the crop

4.3.2 Proline Content

Data regarding Proline content in leaves recorded at different growth stages are given in Table 4.7 and illustrated as Fig 4.7.

At 60 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal) . As maximum Proline content was recorded in treatment T_7 (1.22) which was followed by T_1 (1.00), T_2 (0.77), T_6 (0.67), T_4 (0.42) and T_5 (0.29), while minimum Proline content was observed in T_3 (0.27).

At 90 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal) . As maximum Proline content was recorded in treatment T_7 (1.46) which was at par with T_6 (1.36), followed by T_1 (1.29), T_4 (0.75), T_5 (0.71) and T_2 (0.65), while minimum Proline content was observed in T_3 (0.49).

Table .4.7: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Proline Content at different stages of the crop

Treatment	30DAS	60DAS	Mean
T_1 Control (untreated)	1.00	1.29	1.14
T_2Nanocombi @ 10mgL^{-1}	0.77	0.65	0.71
T_3 Nanocombi @ 20mgL^{-1}	0.27	0.49	0.38
T_4 Nanocombi @ 30mgL^{-1}	0.42	0.75	0.58
T_5 Nanocombi @ 40mgL^{-1}	0.29	0.71	0.5
T_6 Nanocombi @ 50mgL^{-1}	0.67	1.36	1.35
T_7 Nanocombi @ 60mgL^{-1}	1.22	1.46	1.34
S.Em	0.0553	0.0428	
CD(0.05)	0.1703	0.1318	

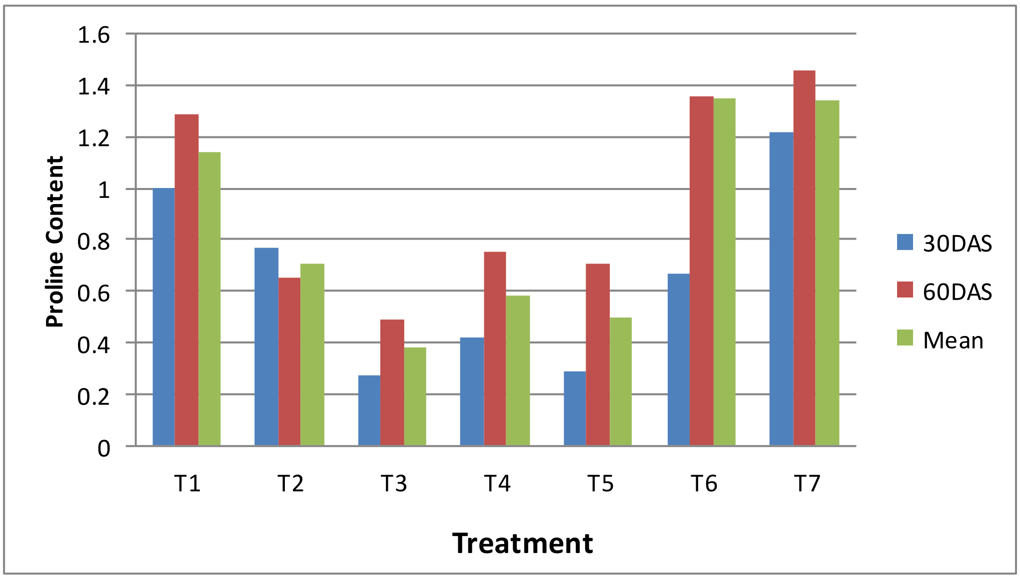


Fig.4.7: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Proline Content at different stages of the crop

4.4 Yield & yield attributes

4.4.1 Plant Height

Plant Height of Ashwagandha crop showed increasing pattern at different growth stages due to foliar spray of nano-micronutrients which is presented and illustrated in Table 4.8 and Fig. 4.8 respectively.

At 30 DAS

The results showed that maximum plant height was obtained in T_3 (5.60) which was followed by T_4 (4.88), T_6 (4.76), T_5 (4.53), T_7 (4.00) and T_2 (3.24), while minimum plant height seen in T_1 (2.61).

At 60 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum plant height was recorded in treatment T_5 (34.30) which was at par with T_4 (32.73), followed by T_6 (29.60), T_2 (27.33), T_7 (26.40), and T_3 (26.40), while minimum plant height was noticed in T_1 (25.23).

At 90 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum plant height was recorded in treatment T_5 (38.95) which was followed by T_4 (36.43), T_6 (35.34), T_1 (33.30), T_7 (32.33), and T_2 (31.97), while minimum plant height was noticed in T_3 (29.43).

At 120 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum plant height was recorded in treatment T_5 (47.43) which was at par with T_4 (46.50), followed by T_7 (43.70), T_3 (43.50), T_6 (42.40), and T_2 (42.30), while minimum plant height was observed in T_1 (41.80).

At Harvest

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum plant height was recorded in treatment T_5 (45.73) which was followed by T_4 (43.63), T_3 (42.37), T_2 (41.47), T_6 (38.37), and T_1 (37.30), while minimum plant height was observed in T_7 (36.77).

Table.4.8: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Plant Height at different stages of the crop

Treatment	30DAS	60DAS	90DAS	120DAS	At Harvest	Mean
T_1 Control (untreated)	2.61	25.23	33.30	41.80	37.30	28.04
T_2 Nanocombi @ 10mgL^{-1}	3.24	27.33	31.97	42.30	41.47	29.26
T_3 Nanocombi @ 20mgL^{-1}	5.60	26.40	29.43	43.50	42.37	29.46
T_4 Nanocombi @ 30mgL^{-1}	4.88	32.73	36.43	46.50	43.63	32.83
T_5 Nanocombi @ 40mgL^{-1}	4.53	34.30	38.95	47.43	45.73	34.18
T_6 Nanocombi @ 50mgL^{-1}	4.76	29.60	35.34	42.40	38.37	30.09
T_7 Nanocombi @ 60mgL^{-1}	4.00	26.40	32.33	43.70	36.77	28.64
S.Em±	0.1991	0.6170	0.5081	0.7943	0.6223	
CD(0.05)	0.6134	1.9010	1.5656	2.4474	1.9175	

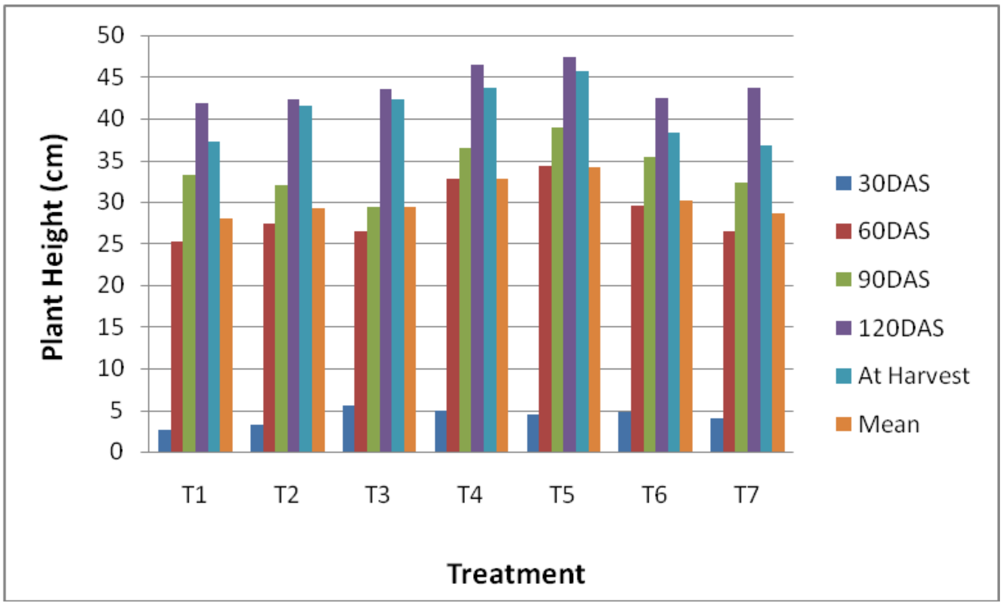


Fig.4.8: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Plant Height at different stages of the crop

4.4.2 Number of branches plant^{-1}

Number of branches in Ashwagandha crop showed increasing pattern at different growth stages due to foliar spray of nano-micronutrients which was presented and illustrated in Table 4.9 and Fig. 4.9 respectively.

At 60 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As highest number of branches were recorded in treatment T_5 (3.00) which was at par with T_4 (2.93), T_6 (2.87), T_7 (2.77) and T_3 (2.63), followed by and T_2 (2.30), while minimum number of branches was noticed in T_1 (1.67).

At 90 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) on Ashwagandha crop (*Withania somnifera* (L.) Dunal) showed significant effect as highest number of branches were observed in treatment T_5 (5.47) which was followed by T_4 (4.67), T_6 (4.63), T_2 (4.53), T_1 (4.43), and T_7 (4.27), while minimum number of branches was observed in T_3 (3.80).

At 120 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) on Ashwagandha crop (*Withania somnifera* (L.) Dunal) showed significant effect as highest number of branches were observed in treatment T_5 (6.00) which was followed by T_4 (5.37), T_6 (5.20), T_2 (4.80), T_3 (4.80), and T_7 (4.77), while minimum number of branches was observed in T_1 (4.40).

At Harvest

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As highest number of branches were recorded in treatment T_5 (5.07) which was at par with T_4 (4.77), followed by T_6 (4.53), T_3 (4.07), T_2 (3.70), while minimum number of branches was observed in T_3 and T_7 (3.57).

Table.4.9: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on number of branches per plant at different stages of the crop

Treatment	30DAS	60DAS	90DAS	120DAS	At Harvest	Mean
T_1 Control (untreated)	0.00	1.67	4.43	4.40	3.57	2.81
T_2 Nanocombi @ 10mgL^{-1}	0.00	2.30	4.53	4.80	3.70	3.06
T_3 Nanocombi @ 20mgL^{-1}	0.00	2.63	3.80	4.80	4.07	3.06
T_4 Nanocombi @ 30mgL^{-1}	0.00	2.93	4.67	5.37	4.77	3.54
T_5 Nanocombi @ 40mgL^{-1}	0.00	3.00	5.47	6.00	5.07	3.90
T_6 Nanocombi @ 50mgL^{-1}	0.00	2.87	4.63	5.20	4.53	3.44
T_7 Nanocombi @ 60mgL^{-1}	0.00	2.77	4.27	4.77	3.57	3.07
S.Em±	0.0000	0.2249	0.1992	0.1846	0.1745	
CD(0.05)	0.0000	0.6930	0.6138	0.5688	0.5377	

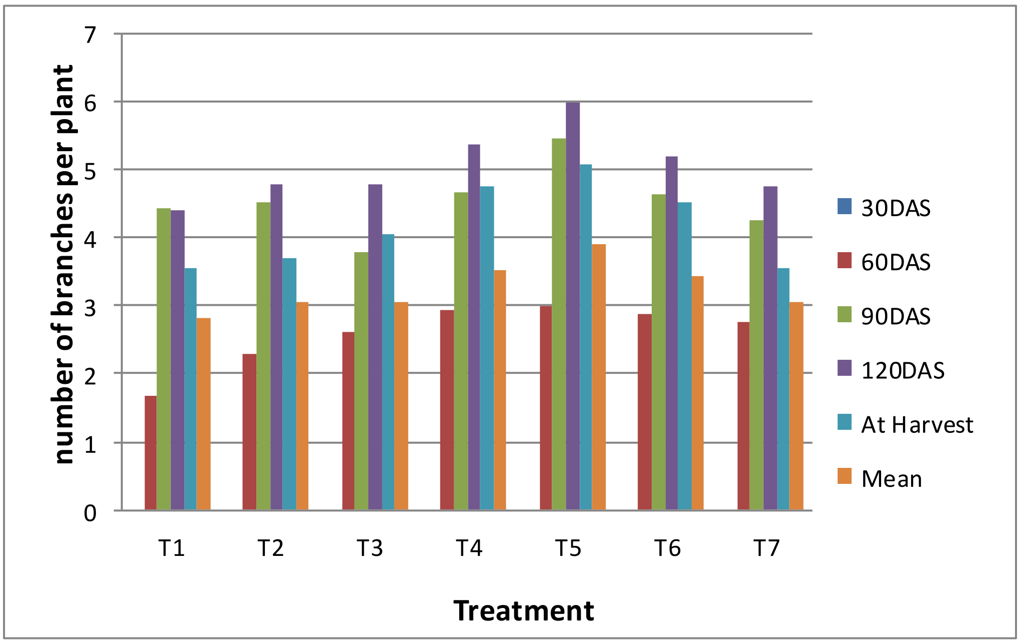


Fig.4.9: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on number of branches per plant at different stages of the crop.

4.4.3 Leaf biomass

Leaf biomass of Ashwagandha crop showed significant increment at different growth stages due to foliar spray of nano-micronutrients which is presented and illustrated in Table 4.10 and Fig. 4.10 respectively.

At 30 DAS

The results showed that maximum leaf biomass was observed in T_3 (128.17) which was followed by T_5 (111.47), T_4 (82.90), T_6 (79.33), T_7 (68.67) and T_2 (58.77), while minimum leaf biomass seen in T_1 (33.70).

At 60 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum leaf biomass was recorded in treatment T_5 (152.00) which was at par with T_4 (145.17) & T_3 (137.63), followed by T_7 (132.70), T_6 (126.97) and T_2 (122.73), while minimum leaf biomass was noticed in T_1 (121.60).

At 90 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum leaf biomass was noticed in treatment T_4 (372.94) which was followed by T_5 (335.65), T_3 (318.89), T_6 (285.21), T_7 (255.52) and T_2 (241.10), while minimum leaf biomass was noticed in T_1 (236.39).

At 120 DAS

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) showed significant effect on Ashwagandha crop (*Withania somnifera* (L.) Dunal). As maximum leaf biomass was observed in treatment T_4 (267.12) which was at par with T_5 (256.98), followed by T_3 (241.39), T_6 (226.65), T_7 (221.90), and T_2 (207.35), while minimum leaf biomass was observed in T_1 (204.40).

At Harvest

Foliar spray of Nano-micronutrients (Zn + Mn + Cu) on Ashwagandha crop (*Withania somnifera* (L.) Dunal) showed significant effect as maximum leaf biomass was observed in treatment T_4 (232.10) which was followed by T_5

(219.67), T_3 (215.25), T_6 (203.04), T_7 (190.28), and T_2 (187.79), while minimum leaf biomass was observed in T_1 (184.56).

Table.4.10: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Leaf biomass at different stages of the crop

Treatment	30DAS	60DAS	90DAS	120DAS	At Harvest	Mean
T_1 Control (untreated)	33.70	121.60	236.39	204.40	184.56	156.13
T_2Nanocombi @ 10mgL^{-1}	58.77	122.73	241.10	207.35	187.79	163.54
T_3 Nanocombi @ 20mgL^{-1}	128.17	137.63	318.89	241.39	215.25	208.26
T_4 Nanocombi @ 30mgL^{-1}	82.90	145.17	372.94	267.12	232.10	220.04
T_5 Nanocombi @ 40mgL^{-1}	111.47	152.00	335.65	256.98	219.67	215.15
T_6 Nanocombi @ 50mgL^{-1}	79.33	126.97	285.21	226.65	203.04	184.24
T_7 Nanocombi @ 60mgL^{-1}	68.67	132.70	255.52	221.90	190.28	173.81
S.Em±	6.5189	5.9558	7.5769	6.3784	3.6045	
CD(0.05)	20.0867	18.3515	23.3466	19.6538	11.1067	

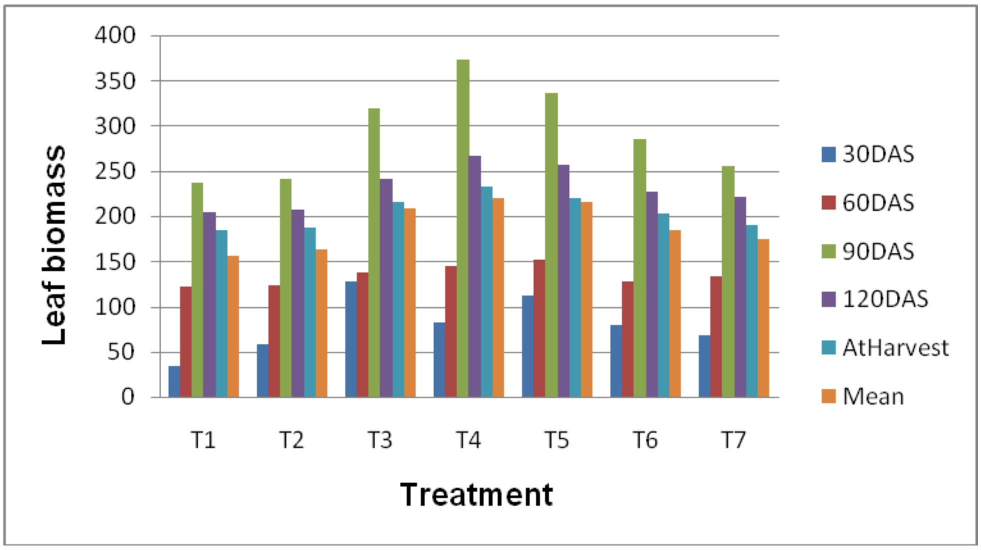


Fig.4.10: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Leaf biomass at different stages of the crop

4.4.4 Number of seeds pod^{-1}plant^{-1}

Data regarding number of seeds pod^{-1}plant^{-1} was recorded are given in Table 4.11 and illustrated in Fig 4.11 Number of seeds pod^{-1}plant^{-1} recorded shown significant effect as influenced by foliar spray of nano-micronutrients on Ashwagandha crop. The results revealed that maximum number of seeds pod^{-1}plant^{-1} were obtained in T_3 (36.67) which was followed by T_4 (30.73), T_5 (24.97), T_6 (22.60), T_2 (21.50) and T_7 (18.73), while minimum number of seeds pod^{-1}plant^{-1} were seen in T_1 (16.37).

Table.4.11: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on number of seeds pod^{-1}plant^{-1}of ashwagandha crop .

Treatment	Seed/pod /plant
T_1 Control (untreated)	16.37
T_2 Nanocombi @ 10mgL^{-1}	21.50
T_3 Nanocombi @ 20mgL^{-1}	36.67
T_4 Nanocombi @ 30mgL^{-1}	30.73
T_5 Nanocombi @ 40mgL^{-1}	24.97
T_6 Nanocombi @ 50mgL^{-1}	22.60
T_7 Nanocombi @ 60mgL^{-1}	18.73
S.Em±	1.4645
CD(0.05)	4.5124

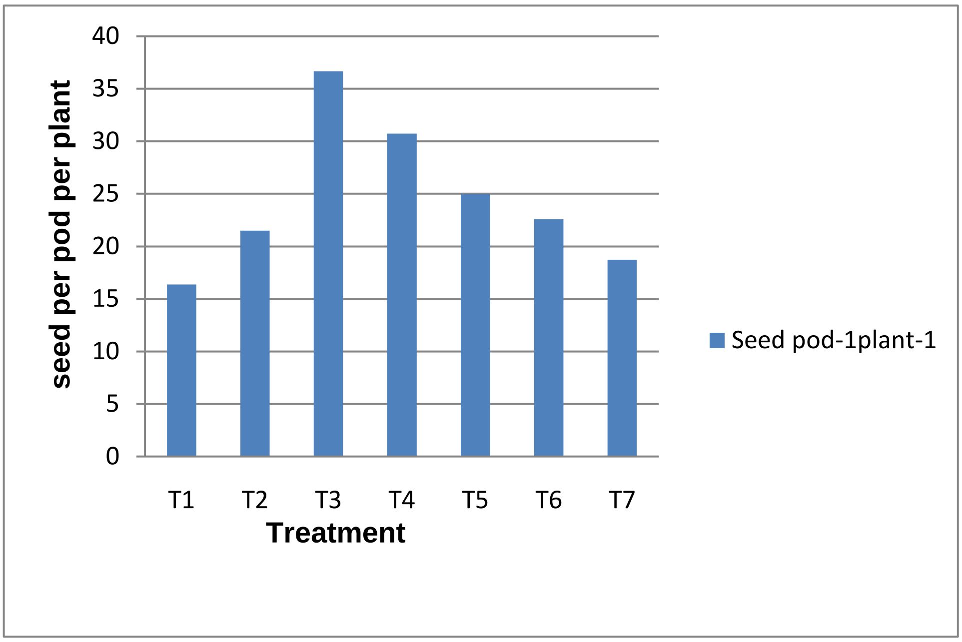


Fig.4.11: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on number of seeds pod^{-1}plant^{-1}of ashwagandha crop.

4.4.5 Root parameters

Root length

Data regarding root length was recorded and was given in Table 4.12 and illustrated in Fig 4.12 Root length recorded shown significant effect as influenced by foliar spray of nano-micronutrients on Ashwagandha crop. The results showed that maximum root length was obtained in T_5 (20.27) which was at par with T_4 (19.40), followed by T_6 (17.83), T_3 (17.77), T_7 (17.53) and T_2 (15.77), while minimum root length was seen in T_1 (15.30).

Root Diameter

Data regarding root diameter obtained was given in Table 4.12 and illustrated in Fig 4.12 Root diameter recorded shown significant effect as influenced by foliar spray of nano-micronutrients on Ashwagandha crop. The results showed that maximum root diameter was obtained in T_5 (11.20) which was followed by T_4 (10.70), T_3 (10.17), T_7 (10.20), T_7 (10.10) and T_2 (9.63) , while minimum root diameter was seen in T_1 (9.33).

Root Dry Weight

Data regarding root dry weight recorded was given in Table 4.12 and illustrated in Fig 4.12 Root dry weight recorded shown significant effect as influenced by foliar spray of nano-micronutrients on Ashwagandha crop. The results showed that maximum root dry weight was obtained in T_5 (12.43) which was at par with T_4 (11.94), followed by T_2 (11.33), T_6 (11.30), T_7 (10.47) and T_3 (10.37) , while minimum root dry weight was seen in T_1 (9.30).

Table.4.12: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Root yield parameters of ashwagandha crop

Treatment	Root length (cm)	Root Diameter(mm)	Dry weight (gplant^{-1})
T_1 Control (untreated)	15.30	9.33	9.30
T_2Nanocombi @ 10mgL^{-1}	15.77	9.63	11.33
T_3 Nanocombi @ 20mgL^{-1}	17.77	10.17	10.37
T_4 Nanocombi @ 30mgL^{-1}	19.40	10.70	11.94
T_5 Nanocombi @ 40mgL^{-1}	20.27	11.20	12.43
T_6 Nanocombi @ 50mgL^{-1}	17.83	10.10	11.30
T_7 Nanocombi @ 60mgL^{-1}	17.53	10.20	10.47
S.Em±	0.3400	0.1335	0.2405
CD(0.05)	1.0476	0.4115	0.7410

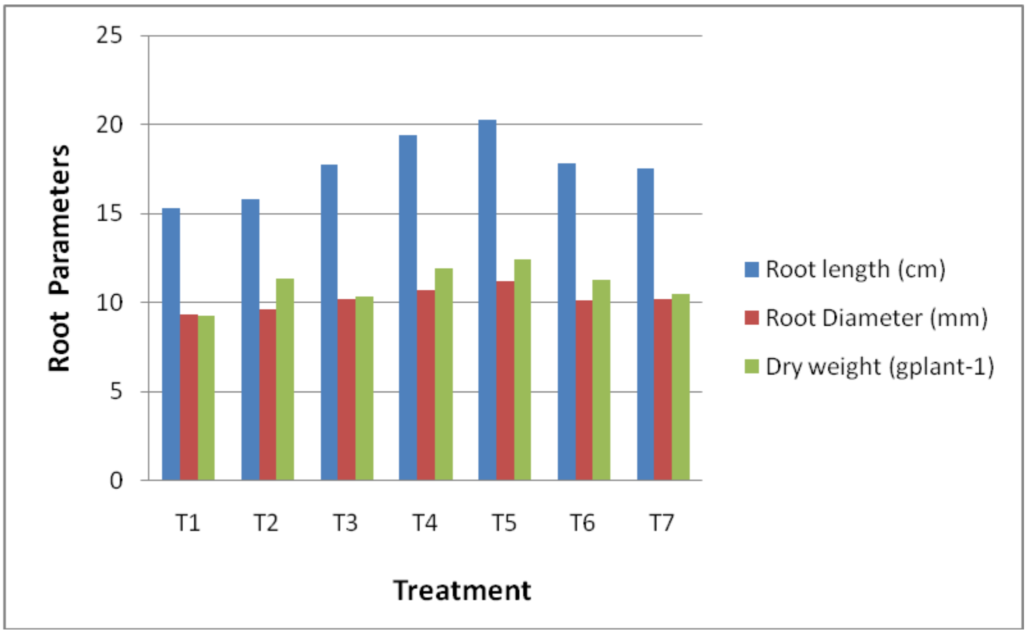


Fig.4.12: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Root yield parameters of ashwagandha crop

4.4.6 Yield (leaf, root and seed yield)

Leaf Yield

The data revealed that leaf yield was recorded and was given in Table 4.13 and illustrated in Fig 4.13 Leaf yield recorded shown significant effect as influenced by foliar spray of nano-micronutrients on Ashwagandha crop. The results showed that maximum leaf yield

was obtained in T_4 (478.05) which was at par with T_5 (461.37), followed by T_3 (438.01), T_5 (410.35), T_2 (350.77) and T_1 (323.55), while minimum leaf yield were seen in T_7 (310.28).

Root Yield

The data revealed that leaf yield was recorded and was given in Table 4.13 and illustrated in Fig 4.13 Root yield recorded shown significant effect as influenced by foliar spray of nano-micronutrients on Ashwagandha crop. The results revealed that maximum root yield

were obtained in T_5 (445.00) which was followed by T_4 (422.00), T_6 (405.67), T_3 (385.33), T_2 (330.33) and T_7 (321.00), while minimum root yield was seen in T_1 (287.00).

Seed Yield

The data revealed that leaf yield was recorded and was given in Table 4.13 and illustrated in Fig 4.13 Seed yield recorded shown significant effect as influenced by foliar spray of nano-micronutrients on Ashwagandha crop. The results revealed that maximum seed yield

were obtained in T_3 (931.21) which was at par with T_4 (866.46), followed by T_5 (762.43), T_2 (759.32), T_6 (733.39) and T_7 (717.09), while minimum seed yield were seen in T_1 (681.59).

Table.4.13: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Yield parameters of ashwagandha crop.

Treatment	Leaf yield (kg ha^{-1})	Root Yield (kg ha^{-1})	Seed Yield (kg ha^{-1})
T_1 Control (untreated)	323.55	287.00	681.59
T_2 Nanocombi @ 10mgL^{-1}	350.77	330.33	759.32
T_3 Nanocombi @ 20mgL^{-1}	438.01	385.33	931.21
T_4 Nanocombi @ 30mgL^{-1}	478.05	422.00	866.46
T_5 Nanocombi @ 40mgL^{-1}	461.37	445.00	762.43
T_6 Nanocombi @ 50mgL^{-1}	410.35	405.67	733.39
T_7 Nanocombi @ 60mgL^{-1}	310.28	321.00	717.09
S.Em±	6.7758	6.5910	29.3404
CD(0.05)	20.8784	20.3090	90.4066

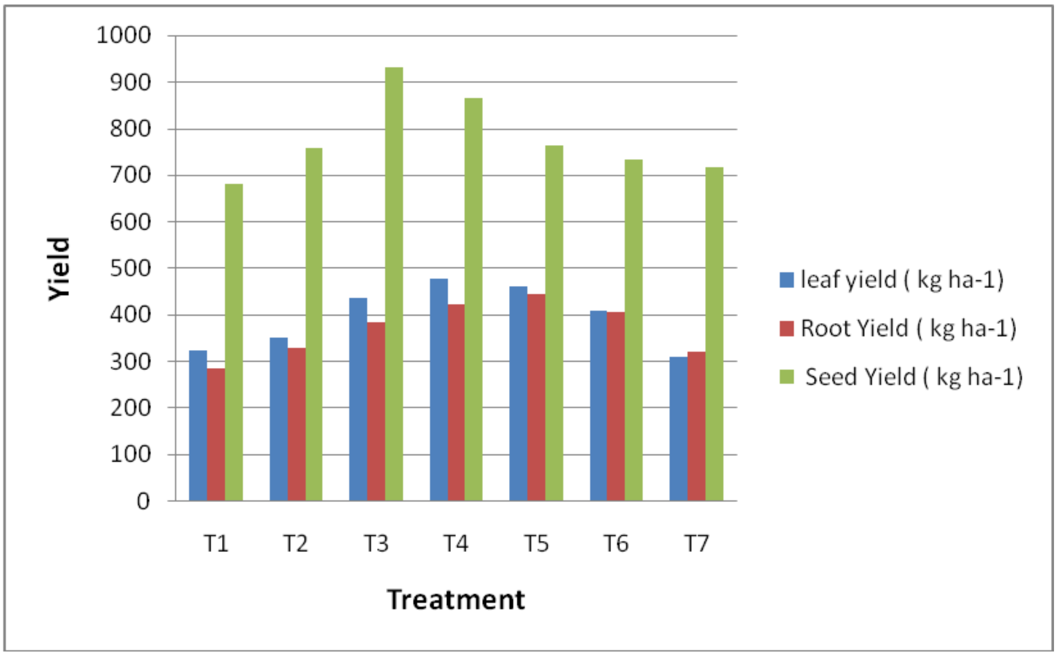


Fig.4.13: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Yield parameters of ashwagandha crop.

4.4.7 Harvest Index

The data showed that Harvest Index of both the economical part (root as well as seed) was recorded and were presented in Table 4.14 and illustrated in Fig 4.14 Harvest Index recorded shown significant effect as influenced by foliar spray of nano-micronutrients on Ashwagandha crop. The results revealed that maximum Harvest Index$_{(root)}$ was obtained in T_5 (15.22) which was at par with T_6 (14.67), followed by T_4 (14.20), T_2 (14.15), T_1 (12.77) and T_7 (12.44), while minimum Harvest Index$_{(root)}$ were seen in T_3 (12.39), while maximum Harvest Index$_{(seed)}$ was obtained in T_3 (31.01) which was at par with T_2 (30.73), followed by T_4 (29.96), T_7 (28.01), T_1 (27.53) and T_5 (26.83), while minimum Harvest Index$_{(seed)}$ were seen in T_6 (26.43).

Table.4.14: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Harvest Index of ashwagandha crop.

Treatment	Harvest Index (HI%) root	Harvest Index (HI%)seed
T_1 Control (untreated)	12.77	27.53
T_2 Nanocombi @ 10mgL^{-1}	14.15	30.73
T_3 Nanocombi @ 20mgL^{-1}	12.39	31.01
T_4 Nanocombi @ 30mgL^{-1}	14.20	29.96
T_5 Nanocombi @ 40mgL^{-1}	15.22	26.83
T_6 Nanocombi @ 50mgL^{-1}	14.67	26.43
T_7 Nanocombi @ 60mgL^{-1}	12.44	28.01
S.Em±	0.2870	0.2994
CD(0.05)	0.8843	0.9225

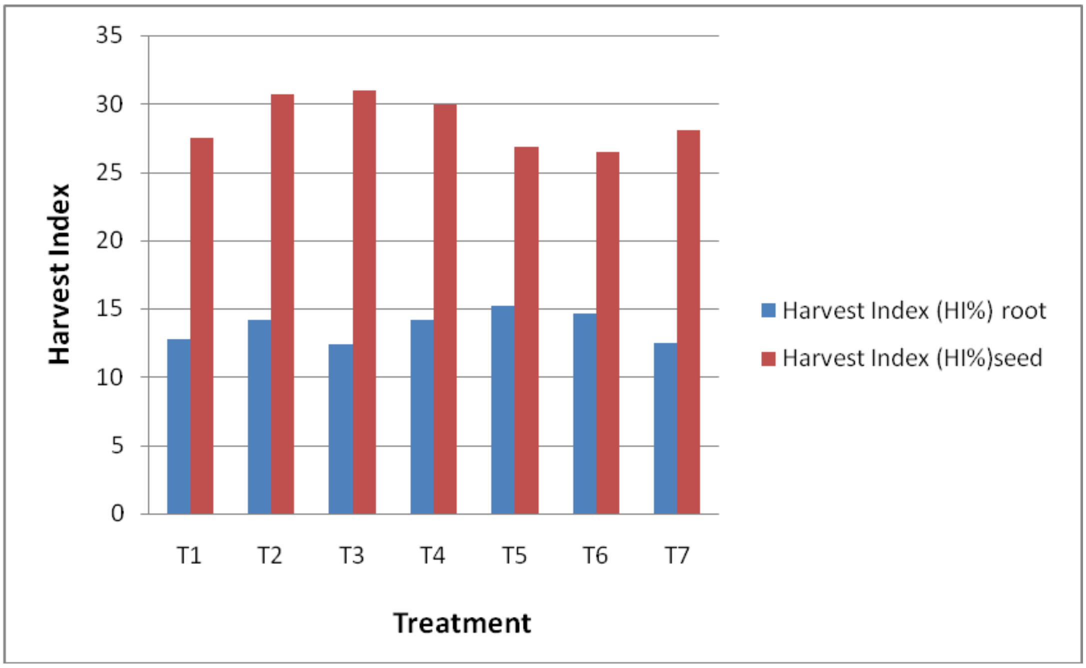


Fig.4.14: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Harvest Index of ashwagandha crop.

4.5 Active ingredient analysis

4.5.1 Withanolide A

The data showed that Withanolide A was recorded and was given in Table 4.15 and illustrated in Fig 4.15 Withanolide A content was significantly influenced by foliar spray of nano-micronutrients on Ashwagandha crop. The maximum Withanolide A (0.13%) was obtained in T_5 which was followed by 0.08% and 0.07% in T_4 and T_2 respectively. The minimum Withanolide A 0.06% was seen in T_1.

Table.4.15: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Withanolide A content of ashwagandha crop.

Treatment	Withanolide content in Root(%)
T_1 Control (untreated)	0.06
T_2 Nanocombi @ 10mgL^{-1}	0.07
T_3 Nanocombi @ 20mgL^{-1}	0.07
T_4 Nanocombi @ 30mgL^{-1}	0.08
T_5 Nanocombi @ 40mgL^{-1}	0.13
T_6 Nanocombi @ 50mgL^{-1}	0.07
T_7 Nanocombi @ 60mgL^{-1}	0.07
S.Em±	0.0132
CD(0.05)	0.0408

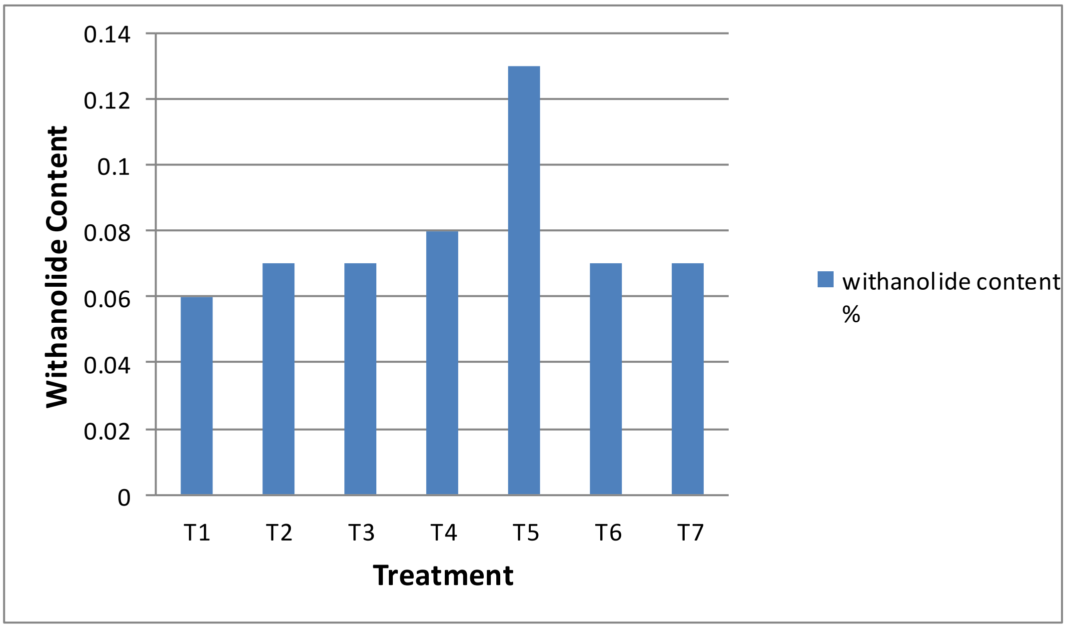
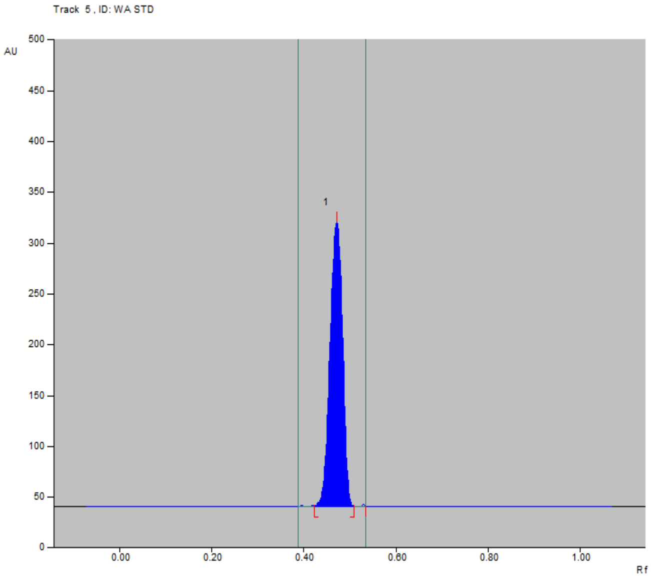
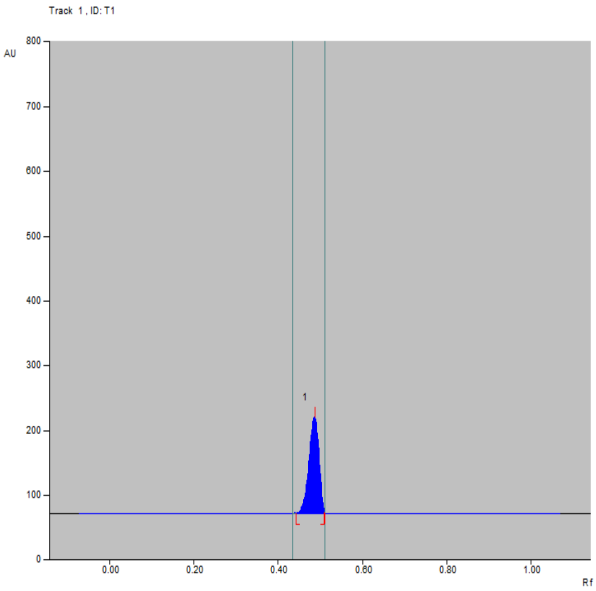


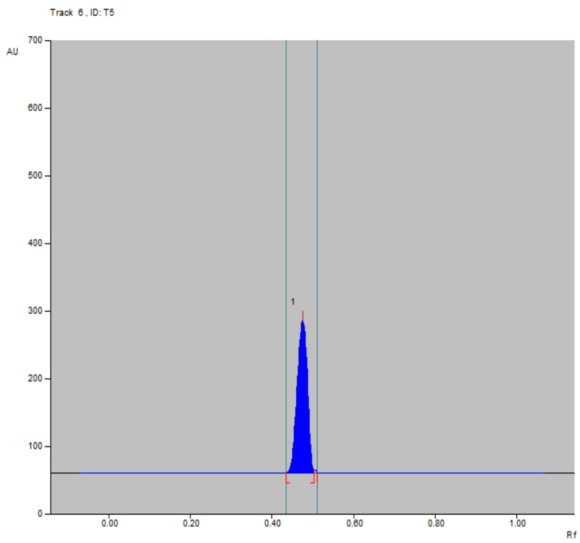
Fig.4.15: Effect of foliar spray of Nanocombi (Zn+ Mn+Cu) on Withanolide A content of ashwagandha crop.



Withanolide A Standard



T_1 Control (untreated)



T_5 Nanocombi @ 40mgL^{-1}

DISCUSSION

Ashwagandha is one the most important medicinal crops cultivated in Indian subcontinent. Among the different factors which are responsible for higher yield and better quality, nutrients (macro as well as micro) also plays major role in enhancing the growth and quality of the produce.The present research work entitled **"Optimization of Nano-micronutrients for phenology, growth, productivity and quality of *Withania somnifera* (L.) Dunal."** was carried out during late *Kharif* season (August 2021- March 2022) to assess the effect of Nano-micronutrients (Zn + Mn + Cu) on various aspects of Ashwagandha crop.

Results obtained during the experiment were discussed in light of finding by previous scientists with proper justifications under the following heads for *Withania somnifera*:

5.1 Phenology

5.2 Physiology

5.3 Biochemistry

5.4 Yield and yield Attributes

5.5 Accumulation of active ingredient.

5.1 Phenology

Phenology of any crop is manifestation of their developmental stages in terms of days taken to achieve specific developmental stages. It gives insight into time and duration of these stages from germination to physical maturity. Long duration in terms of days during vegetative stages of phenology favors higher biomass of vegetative parts. On the other hand, early commencement of reproductive stages of phenology promotes early maturity of crop and to avoid crop from stresses during late maturity.

Use of micronutrients as nano-fertilizer recorded decreased number of days to achieve primary & secondary branching, floral initiation, and 50% flowering, seed formation and fruit maturity. Hence use of nano-micronutrients promoted earliness in all the phenological stages. It may be due to the reason that micronutrients play a central role in plant metabolism maintenance, growth and production, stress tolerance and disease resistance (Shahzad and Amtmann, 2017).

Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave most earliness in primary& secondary branching in Ashwagandha when compared with control treatment (i.e. only water spray).It is due to the reason that zinc is an essential component and activator of many enzymes which take part in auxin biosynthesis and photosynthesis and therefore plays a major role in plant growth and development. (Romheld and Marscher 1991). It was supported by Yadav *et al*. (2001) as they also observed maximum branching due to application of micronutrients.

Nano-micronutrients (Zn + Mn + Cu) @ 20mgL^{-1} gave most earliness in initiation of flowers and attaining 50% flowering in Ashwagandha when compared with control treatment (i.e. only water spray). It was contrary to the research work conducted by Singh *et al*. (1989) as they applied zinc sulphate @ 20, 40 kg/ha and 20 kg ZnSO4/ha + 0.5 % as foliar spray before flowering, but they did not show any difference on days to 50 % flowering. However, flowering was little early in control (55.52 days) than zinc treatment at 20 kg $ZnSO_4$, +0.5% $ZnSO_4$ in chilli.

Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave most earliness in fruit initiation and attaining 50% fruiting in Ashwagandha when compared with control treatment (i.e. only water spray).

The reason behind this may be the application of Cu as a micronutrient, as Cu plays an essential role in many processes such as pollen formation, pollen viability, pollination and lipid desaturation.

Nano-micronutrients (Zn + Mn + Cu) @ 20mgL^{-1} gave most earliness in seed formation in Ashwagandha when compared with control treatment (i.e. only water spray).

Nano-micronutrients (Zn + Mn + Cu) @ 20mgL^{-1} gave most earliness in fruit maturity in Ashwagandha when compared with control treatment (i.e. only water spray).

5.2 Physiology

Physiology of growth and productivity of any crop in terms of growth parameters, source-sink relationship as well as biomass accumulation is greatly affected by nutrient management and nutrient use efficiency of that particular crop. In ashwagandha crop, root and leaf biomass is the major economic part medicinally. Therefore, physiological parameters of growth are directly influenced by the dose of micronutrients (Zn + Mn + Cu).

Use of micronutrients as nano-fertilizer resulted in maximum number of leaves per plant, highest LAI and highest LAD at different growth stages of the *Withania somnifera*.

Nano-micronutrients (Zn + Mn + Cu) @ 20mgL^{-1} gave maximum number of leaves at 60DAS, while Nano-micronutrients (Zn + Mn + Cu) @ 40mgL^{-1} gave highest result at 90DAS but Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave maximum number of leaves at 120DAS and at harvest stage of Ashwagandha crop as compared to control treatment (i.e. only water spray). It is relevant to the findings of Rahman *et al*. (2011), Tawab *et al*. (2015) as they also observed highest number of leaves with the application of Zn at different concentrations for various solanaceous crops.

Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave maximum LAI at 90DAS, while Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave highest LAI at 120DAS but Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave maximum LAI at harvest stage of Ashwagandha crop as compared to control treatment (i.e. only water spray). It is in support with the findings of Elbaky *et al*. (2006) and Banerjee *et al*. (2016) as they also applied Zinc at different concentration on different solanaceous crops and noted the highest LAI per plant.

Nano-micronutrients (Zn + Mn + Cu) @ 20mgL^{-1} gave maximum LAD at 30-60 DAS, while Nano-micronutrients (Zn + Mn + Cu) @ 20mgL^{-1} gave highest LAD at 60-90DAS but Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave maximum LAD at 90-120DAS and Nano-micronutrients (Zn + Mn + Cu) @ 40mgL^{-1} gave maximum LAD at 120-harvest stage of Ashwagandha crop as compared to control treatment (i.e. only water spray). It may be due to the slow releasing action of nanofertilizers which provide nutrients to the crop throughout the life cycle which leads to persistence of leafiness.

5.3 Biochemistry

Biochemical traits of any crop in terms of chlorophyll content, proline content is greatly affected by nutrient management of that particular crop. Hence, use of micronutrients as nano-fertilizer at definite concentrations resulted in maximum CCI per plant at different growth stages of the crop.

Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave maximum CCI at 60DAS, while Nano-micronutrients (Zn + Mn + Cu) @ 20mgL^{-1} gave highest CCI at 90DAS but Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave maximum CCI at 120 and at harvest stage of Ashwagandha crop as compared to control treatment having no use of Nano-fertilizer (i.e. only water spray).). It is in support with the findings of Salam *et al*. (2011),
Razek *et al*. (2013), Lalelou et al. (2013), Sharma (2012) as they also noted the effect of micronutrients in enhancing the chlorophyll content of various cucurbits and crucifers.

Nano-micronutrients (Zn + Mn + Cu) @ 60mgL^{-1} gave maximum Proline content at 60DAS, while Nano-micronutrients (Zn + Mn + Cu) @ 60mgL^{-1} gave at 90DAS maximum Proline content. It may be due to the reason that at definite concentration proline level is optimum but after increasing the concentration it leads to toxicity, resulting to abiotic stresses response by the crop.

5.4 Yield and yield attributes

Yield and yield attributes of any crop are highly influenced by the foliar application of nano-micronutrients individually as well as in combinations as definite doses of nano-micronutrients leads to maximum plant height, number of branches per plant, maximum leaf biomass at different growth stages of the crop, also maximum number of seeds per pod per plant, maximum root length, root diameter and root dry weight, and maximum yield and harvest index been recorded due to application of micronutrient as a nano-fertilizer.

Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave highest plant height at 60DAS, while Nano-micronutrients (Zn + Mn + Cu) @ 40mgL^{-1} gave highest plant height at 90DAS but Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave maximum plant height at 120DAS, while Nano-micronutrients (Zn + Mn + Cu) @ 40mgL^{-1} gave highest plant height at harvest stage of Ashwagandha crop as compared to control treatment (i.e. only water spray). It is relevant to the findings of Jana *et al*. (1987), Dod *et al.* (1989), Hussani *et al*. (1989), Romheld and Marscher (1991), Bose and Tripathi (1996), Kumar *et al*. (2008), Rahman *et al*. (2011), Ali *et al*. (2015) .It is due to the role of zinc in auxin synthesis and association of boron for development of cell wall and cell differentiation that plays major role in root and shoot growth in plants. Application of zinc at absolute concentration also provides better root system for the plant for absorption of water and other nutrients dissolved in it.

Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave maximum number of branches per plant at 60DAS, while Nano-micronutrients (Zn + Mn + Cu) @ 40mgL^{-1} gave maximum number of branches per plant at 90DAS and 120DAS, while Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave maximum number of branches per plant at harvest stage of Ashwagandha crop as compared to treatment having no use of Nano-fertilizer (i.e. only water spray). It is relevant to the findings of Bose and Tripathi (1996), Yadav *et al*. (2001), Khasti and Rana (2012), Naga *et al*. (2013), Ali *et al*. (2015). It may be due to the fact that zinc activates enzyme activities and increases the auxin synthesis which plays essential role in the growth and development of plant.

Nano-micronutrients (Zn + Mn + Cu) @ 20mgL^{-1} gave maximum leaf biomass at 60DAS, while Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave maximum leaf biomass at 90DAS and 120DAS, while Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} gave maximum leaf biomass at harvest stage of Ashwagandha crop as compared to treatment having no use of Nano-fertilizer (i.e. only water spray). It is relevant to the findings of Puzina (2004), Rahman *et al*. (2011), Harris and Mathuma (2015).

Nano-micronutrients (Zn + Mn + Cu) @ 20mgL^{-1} gave maximum number of seeds per pod per plant in Ashwagandha as compared to treatment having no use of Nano-fertilizer (i.e. only water spray). It is relevant to the findings of Nadergoli *et al*. (2011), Shabanzadesh and Galavi (2011) as they found significant effect of micronutrient application on black cumin.

Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} resulted in maximum root length, whereas Nano-micronutrients (Zn + Mn + Cu) @ 40mgL^{-1} resulted in maximum root diameter, also Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} resulted in maximum root dry weight as compared to treatment having no use of Nano-fertilizer (i.e. only water spray). It is relevant to the findings of Harris and Mathuma (2015), Raghav and Singh (2003) it may be due to the role of zinc in auxin synthesis and association of boron for development of cell wall and cell differentiation that plays major role in root and shoot growth in plants.

Root is the main useful and marketable economic parts of *Withania* crop. Hence, it is pertinent to test any management input in terms of root yield in order to find out the optimum/best management input. In present experiment, nano-micronutrient combinations of Zn, Mn and Cu were optimized on the basis of their response in increasing root biomass. 40mgL^{-1} of nano-micronutrient combinations (Zn + Mn + Cu) gave significantly maximum root yield (445.00 kg ha^{-1}) as compared to all other treatment of nano-micronutrients. This yield was 55.05% higher than the treatment in which no nano-micronutrient combinations were sprayed. (i.e. only water spray).

30mgL^{-1} of nano-micronutrient combinations (Zn + Mn + Cu) gave significantly maximum leaf yield (478.05 kg ha^{-1}) as compared to all other treatment of nano-micronutrients. This yield was 54.07% higher than the treatment in which no nano-micronutrient combinations were sprayed. (i.e. only water spray).

30mgL^{-1} of nano-micronutrient combinations (Zn + Mn + Cu) gave significantly maximum seed yield (931.21kg ha^{-1}) as compared to all other treatment of nano-micronutrients. This yield was 36.62% higher than the treatment in which no nano-micronutrient combinations were sprayed. (i.e. only water spray). It is relevant to the findings of Bid *et al*. (1992), Ravichandran *et al*. (1995), Raj *et al*. (2001), Pariari *et al*. (2009), Ahmed *et al*. (2011), Kumar *et al*. (2012), Jawad (2016. It may be due to the slow releasing action of nanofertilizers which provide nutrients to the crop throughout the life cycle which leads to availability of nutrients to the crop at seed formation as well at time of fruit maturity. It also helps in better partitioning of assimilates.

Nano-micronutrients (Zn + Mn + Cu) @ 40mgL^{-1} resulted in maximum harvest index $_{(root)}$, whereas Nano-micronutrients (Zn + Mn + Cu) @ 10mgL^{-1} resulted in maximum harvest index $_{(seed)}$ in Ashwagandha as compared to treatment having no use of Nano-fertilizer (i.e. only water spray). It may be due to the reason that micronutrients play a central role in plant metabolism maintenance, growth and production.

5.5 Accumulation of active ingredient

Active ingredients are major molecules responsible for biological (medicinal) activity in human physiology as medicine. More the active ingredient in raw drug adds more value in terms of quality and market value of the raw drug. *Withania somnifera* roots have many biomolecules of alkaloid and steroidal category (Withanolides).

In present experiment spray of nano-micronutrients (Zn + Mn + Cu) of various concentrations in *Withania* crop was tested to find variation in Withanolide A content in roots.

Spray of nano-micronutrients (Zn + Mn + Cu) @ 40mgL^{-1} resulted in maximum accumulation of Withanolide A (0.13) as compared to all other concentrations of nano-micronutrients along with no spray of nano-micronutrients (i.e. only water spray). This concentration is 116.66% more as compared to no spray of nano-micronutrients.

The increase in Withanolide A in response to nano-micronutrient spray is due to the reason that increased concentration of micronutrients induces the stress in *Withania* crop and resulted in more accumulation of Withanolide A upto the concentration of spray @ 40mgL^{-1}. Beyond this concentration of nano-micronutrients combination, Withanolide A accumulation in root was drastically inhibited due to possible toxic effects of nano-micronutrients for *Withania* crop.

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

The present research work entitled "**Optimization of Nano-micronutrients for phenology, growth, productivity and quality of *Withania somnifera* (L.) Dunal**" was conducted during the *Kharif* session [2021-2022] at Experimental field of AICRP on Medicinal and Aromatic Plants JNKVV, Jabalpur (MP).

The present experiment was conducted in Randomized Block Design with three replications, and the observations were taken taking different phenological parameters viz*.,* days to seedling emergence, days to primary branching, days to secondary branching, days to floral initiation, days to 50% flowering, days to fruit initiation, days to 50% fruiting, days to seed formation, days to 1^{st} fruit maturity and days to 50% maturity. Physiological parameters were number of leaves per plant, leaf area index (LAI), Leaf Area Duration (LAD). Biochemical parameters included were Chlorophyll content index (CCI), proline content. Yield and yield attributes included Plant height (cm), Number of branches plant^{-1}, leaf biomass (kgha^{-1}), Number of seeds pod^{-1} plant^{-1}, root length(cm), root diameter (mm), root dry weight (gplant^{-1}), Leaf yield(kgha^{-1}), Root yield (kgha^{-1}),Seed yield(kgha^{-1}), Harvest Index(%), Withanolide content . The experimental findings are summarized below:

SUMMARY:

- Foliar spray of different concentration of Nano-micronutrients (Zn+Mn+Cu) significantly influenced the phenology of crop, which was evident in decreased number of days to achieve primary and secondary branching, floral initiation, fruit setting and fruit maturity. This study showed that foliar application of Nano-micronutrients (Zn + Mn + Cu) @ 30mgL^{-1} resulted in earliness to primary and secondary branching , fruit initiation and 50% fruiting whereas, Nano-micronutrients (Zn + Mn + Cu) @ 20mgL^{-1} resulted in earliness to floral initiation,50% flowering, seed formation and fruit maturity.

- Foliar spray of Nano-micronutrients (Zn+Mn+Cu) @ 40mgL^{-1} showed highest average number of leaves plant^{-1} (43.85) whereas, foliar spray of Nano-micronutrients (Zn+Mn+Cu) @ 30mgL^{-1} shown highest average Leaf Area Index (2.05). Similarly foliar spray of Nano-micronutrients (Zn+Mn+Cu) @ 40mgL^{-1} showed highest average LAD (65.10).
- Nano-micronutrients (Zn+Mn+Cu) @ 30mgL^{-1} maintained better average Chlorophyll content index (56.94) when compared to other treatments.
- Yield and yield attributes studies showed that foliar spray of Nano-micronutrients (Zn+Mn+Cu) @ 40mgL^{-1} resulted in superior average plant height (34.18cm), maximum average number of branches (3.90), root diameter (11.20mm), root yield (445.00 kg ha^{-1}), Harvest index $_{root}$ (15.22%) whereas, Nano-micronutrients (Zn+Mn+Cu) @ 30mgL^{-1} shown maximum average leaf biomass (220.04 kg ha^{-1}) ,root length (19.40cm), root dry weight(11.94 g plant^{-1}), leaf yield (478.05 kg ha^{-1}) whereas, Nano-micronutrients (Zn+Mn+Cu) @ 20mgL^{-1} shown maximum number of seeds per pod per plant (36.67), seed yield (931.21 kg ha^{-1}) whereas, Nano-micronutrients (Zn+Mn+Cu) @ 10mgL^{-1} shown maximum Harvest index $_{seed}$ (30.73%).
- Active ingredient content studies revealed that foliar spray of Nano-micronutrients (Zn+Mn+Cu) @ 40mgL^{-1} had highest Withanolide content (0.13%).

CONCLUSION

Foliar application of Nano-micronutrients (Zn+Mn+Cu) @ 40mgL^{-1} significantly influenced the number of leaves plant^{-1} (43.85), average LAD (65.10), average plant height (34.18cm), maximum average number of branches (3.90), root diameter (11.20mm), root yield (445.00 kg ha^{-1}), Harvest index $_{root}$ (15.22%), Withanolide A content (0.13%).

Foliar application of Nano-micronutrients (Zn+Mn+Cu) @ 30mgL^{-1} when compared to other treatments maintained higher Leaf Area Index (2.05), average Chlorophyll content index (56.94), average leaf biomass (220.04 kg ha^{-1}) ,root length (19.40cm), root dry weight(11.94 g plant^{-1}), leaf yield (478.05 kg ha^{-1}).

Foliar application of Nano-micronutrients (Zn+Mn+Cu) @ 20mgL^{-1} significantly influenced the number of seeds per pod per plant (36.67), seed yield (931.21 kg ha^{-1}).

Foliar application of Nano-micronutrients (Zn+Mn+Cu) @ 10mgL^{-1} leads to significantly influencing Harvest index $_{seed}$ (30.73%).

Hence, the foliar spray of 40mgL^{-1} of Nano-micronutrients (Zn+Mn+Cu) was found to be superior among all the concentrations, as it enhanced the root yield (445.00 kg ha^{-1}) which is the economic part of the crop, also resulted in maximum Withanolide A content (0.13%), Harvest Index $_{root}$ (15.22%), maximum plant height (34.18cm).

Suggestion for future work:

- The superiority of foliar application of Nano-micronutrients (Zn+Mn+Cu) @ 40mgL^{-1} needs to be verified for root yield and other growth parameters at different locations, also Nano-micronutrients (Zn+Mn+Cu) @ 30mgL^{-1} needs to be verified for leaf yield and other physiological parameters at different locations, Nano-micronutrients (Zn+Mn+Cu) @ 20mgL^{-1} needs to be verified for seed yield at different locations.
- The treatment needs further exploration for yield stability studies under rainfed, late sown conditions.
- Nano-micronutrients individually as well as in combination should also be used as soil application and seed inoculants so as to test growth, productivity and quality of *Withania somnifera* roots in further research work.

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APPENDIX

A. Phenological Parameters of Ashwagandha

Source of variation	D.F.	MSS and F cal at different DAS									
		Days to seedling emergence		Days to primary branching		Days to secondary branching		Days to floral initiation		Days to 50% flowering	
		MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal
Replications	2	1	2.571	1.19047	1.304	1.1904	1.163	0.4285	0.491	2.0476	5.375
Treatment	6	2.41269	6.204	4.0793	4.470	3.7619	3.674	3.2063	3.673	9.4285	24.750
Error	12	0.38888		0.9126		1.0238		0.8730		0.3809	

B. Phenological Parameters of Ashwagandha

Source of variation	D.F.	MSS and F cal at different DAS									
		Days to fruit initiation		Days to 50% fruiting		Days to seed formation		Days to1st fruit maturity		Days to 50% fruit maturity	
		MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal
Replications	2	0.1428	0.222	0.476	0.087	1.3333	1.600	2.0476	2.481	0.0476	0.051
Treatment	6	21.8571	34.000	5.4285	9.913	4.3809	5.257	4.3015	5.212	4.3174	4.610
Error	12	0.6428		0.5476		0.8333		0.8253		0.9365	

C. Physiological Parameters of Ashwagandha for number of leaves per plant.

Source of variation	D.F.	MSS and F cal at different DAS									
		30DAS		60 DAS		90 DAS		120DAS		At Harvest	
		MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal
Replications	2	0.7104	2.014	1.7033	0.843	0.6990	0.398	0.3190	0.45	0.833	3.885
Treatment	6	2.5574	7.251	36.0252	17.834	61.0987	34.745	62.2519	88.690	35.6787	18.933
Error	12	0.3526		2.02		1.7584		0.7019		1.8844	

D. Physiological Parameters of Ashwagandha for LAI

Source of variation	D.F.	MSS and F cal at different DAS									
		30DAS		60 DAS		90 DAS		120DAS		At Harvest	
		MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal
Replications	2	4.7619	0.005	0.02049	0.504	0.2287	0.489	0.0084	0.269	0.2150	2.449
Treatment	6	0.0010	1.155	0.04649	1.143	0.5865	12.546	0.1685	5.330	0.1133	12.903
Error	12	0.00089		0.04066		0.04674		0.03161		0.00878	

E. Physiological Parameters of Ashwagandha for LAD

Source of variation	D.F.	MSS and F cal at different DAS							
		30-60DAS		60-90DAS		90-120 DAS		120-At harvest	
		MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal
Replications	2	2.3562	0.626	3.6395	2.481	0.3838	0.100	4.2297	0.493
Treatment	6	27.1417	7.210	70.6196	48.131	20.1758	5.276	240.3260	28.008
Error	12	3.7646		1.4672		3.8242		8.5805	

F. Biochemical Parameters of Ashwagandha for Chlorophyll content index

Source of variation	D.F.	MSS and F cal at different DAS									
		30DAS		60 DAS		90 DAS		120DAS		At Harvest	
		MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal
Replications	2	2.7586	0.632	0.66257	0.386	0.12917	0.165	0.67491	0.500	0.6646	1.600
Treatment	6	56.6630	12.986	146.625	85.479	61.5222	78.799	35.645	26.384	22.5078	54.175
Error	12	4.363477		1.71533		0.780753		1.35099		0.4156	

G. Biochemical Parameters of Ashwagandha for Proline content

Source of variation	D.F.	MSS and F cal at different DAS			
		30DAS		60DAS	
		MSS	F cal	MSS	F cal
Replications	2	0.0032714	0.357	0.0013905	0.254
Treatment	6	0.3968603	43.324	0.4756111	86.712
Error	12	0.0091603		0.0054849	

H. Yield and Yield Attributes of Ashwagandha for Plant Height

Source of variation	D.F.	MSS and F cal at different DAS									
		30DAS		60 DAS		90 DAS		120DAS		At Harvest	
		MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal
Replications	2	0.2506	0.211	1.6585	1.452	0.1292	0.167	1.16761	0.617	1.9090	1.643
Treatment	6	3.15223	26.511	36.3652	31.846	30.210	39.014	14.3242	7.568	34.8515	29.997
Error	12	0.11890		1.1419		0.7745		1.89261		1.16182	

I. Yield and Yield Attributes of Ashwagandha for number of branches per plant.

Source of variation	D.F.	MSS and F cal at different DAS									
		30DAS		60 DAS		90 DAS		120DAS		At Harvest	
		MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal
Replications	2	0	#DIV/0!	0.2661	1.754	0.15571	1.308	0.333	0.033	0.05190	0.568
Treatment	6	0	#DIV/0!	0.666031	4.389	0.7585	6.372	0.82650	8.085	1.1253	12.320
Error	12	0		0.15174		0.11904		0.10222		0.09134	

J. Yield and Yield Attributes of Ashwagandha for leaf biomass.

Source of variation	D.F.	MSS and F cal at different DAS									
		30DAS		60 DAS		90 DAS		120DAS		At Harvest	
		MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal	MSS	F cal
Replications	2	5.87285	0.046	38.255	0.360	611.0623	3.548	60.6858	0.497	56.33506	1.445
Treatment	6	3020.3682	23.692	36.8298	3.729	8120.076	47.148	1722.7064	14.115	994.508	25.514
Error	12	127.487		106.412		172.2259		122.0521		38.9782	

K. Yield and Yield Attributes of Ashwagandha for Number of seeds per pod per plant.

Source of variation	D.F.	MSS and F cal at different DAS	
		Number of seeds	
		MSS	F cal
Replications	2	5.2633	0.818
Treatment	6	149.557	23.245
Error	12	6.43388	

L. Yield and Yield Attributes of Ashwagandha for root parameters

Source of variation	D.F.	MSS and F cal values					
		Root length		Root diameter		Root dry weight	
		MSS	F cal	MSS	F cal	MSS	F cal
Replications	2	0.6061	1.748	0.149204	2.786	0.55223	3.183
Treatment	6	9.51269	27.434	1..16634	21.804	3.350226	19.311
Error	12	0.34674		0.53492		0.173488	

M. Yield of Ashwagandha

Source of variation	D.F.	MSS and F cal values					
		Leaf Yield		Root Yield		Seed Yield	
		MSS	F cal	MSS	F cal	MSS	F cal
Replications	2	87.6872	0.637	440.0476	3.377	198.6941	0.077
Treatment	6	13810.10	100.266	10346.9683	79.393	23440.448	9.076
Error	12	137.7353		130.32539		2582.56	

N. Harvest Index of Ashwagandha

Source of variation	D.F.	MSS and F cal at different DAS			
		Harvest Index(root)		Harvest Index(seed)	
		MSS	F cal	MSS	F cal
Replications	2	0.33719	0.136	0.08380	0.312
Treatment	6	3.93747	15.935	10.73580	39.927
Error	12	0.21470		0.26888	

O. Active Ingredient content of Ashwagandha

Source of variation	D.F.	MSS and F cal at different DAS	
		Withanolide content	
		MSS	F cal
Replications	2	0.000414	0.786
Treatment	6	0.001744	3.313
Error	12	0.000526	

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For the partial fulfillment of the master‟s degree programme she was allotted a research problem on **"Optimization of Nano-micronutrients for phenology, growth, productivity and quality of *Withania somnifera* (L.) Dunal"** which was successfully conducted by her and being submitted in the form of the thesis.