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Influence of plant growth regulators and nutrients on growth and productivity of chickpea under fluctuating environments

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Abstract

A field experiment was carried out in a split-plot design with three replications during the winter (*rabi*) season of 2022-23 at PGI Farm, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India. In this experiment, "Influence of plant growth regulators and nutrients on growth and productivity in chickpea under fluctuating environments" was studied. The chickpea variety Phule Vikram was used as a test crop. The experiment consisted of two factors, *i.e.*, sowing dates and treatments. The main factor included three sowing dates: second week of November, first week of December and fourth week of December. The sub-factor included eight treatments: Control, foliar applications of Nanourea @ 0.2% at flowering and pod filling stages, foliar applications of Potassium Nitrate @ 2% at flowering and pod filling stages, foliar applications of Boron @ 0.1% at flowering and pod filling stages, foliar applications of Kinetin @ 10 ppm at flowering and pod filling stages, foliar applications of CCC (Chlormequate chloride 50%) @ 30 ppm at flowering and pod filling stages, foliar applications of Ethrel (2-Chloroethyl phosphonic acid 39%) @ 20 ppm at flowering and pod filling stages and foliar applications of NAA (α -Naphthyl Acetic Acid) @ 10 ppm at flowering and pod filling stages. All observations were recorded at different growth stages of the crop and analysis was done as per the standard procedure. The experiment revealed that the second week of November was significantly superior for increasing plant height (66.45 cm), number of secondary branches (13.99), leaf area (10.21 cm²), pods per plant (95.64), seeds per pod (1.62), 100-seed weight (21.05 g), biological yield (6171.81 kg ha⁻¹) and seed yield per hectare (2748.13 kg ha⁻¹) of chickpea compared to the first and fourth weeks of December. The experiment also revealed that the foliar application of Potassium Nitrate @ 2% at flowering and pod filling stages significantly influenced leaf area (10.35 cm²), number of pods per plant (85.81), number of seeds per plant (1.67), 100-seed weight (21.30 g) and seed yield per hectare (2833.67 kg ha⁻¹). The interaction between sowing date (second week of November) and treatment (foliar application of Potassium Nitrate @ 2% at flowering and pod filling stages) was found to be significantly superior in increasing the number of pods per plant (106.58) and seed yield per hectare (3222.60 kg ha⁻¹). The correlation data revealed that the correlation coefficient between morphological and yield-related characteristics with economic yield and biological yield showed a significantly positive correlation (at the 1% level), indicating a strong positive correlation with yield.

Keywords: Chickpea, sowing dates, plant growth regulators, nutrients, correlation

1. Introduction

The chickpea (*Cicer arietinum* L.) is an annual legume of the Fabaceae family, subfamily Faboideae (Toker *et al.*, 2014) [26]. Chickpea is the third most important legume crop globally, following beans and peas in terms of production and significance. India is the largest producer of chickpea, occupying an area of about 33.21 lakh hectares with a total production of 25.77 lakh tons and a yield of 776 kg ha⁻¹, contributing 73% of the area and 75% of the production among pulses (INDIASTAT, 2023) [1]. The November-April period is considered the ideal time for chickpea cultivation, with 60-65% of chickpeas in India grown during this season. The area of chickpea grown in late-sown conditions is increasing, especially in northern and central India, due to its inclusion in new cropping systems and more intense sequential cropping practices. This leads to prolonged exposure of chickpea to high temperatures. The average maximum temperature required for chickpea ranges from 21 to 29 °C during the day and 15 to 25 °C during the night (Devasirvatham *et al.*, 2013) [5].

Temperature is an important constraint for chickpea crops. Cold temperatures during early growth stages slow down vegetative development, leading to poor plant establishment (Bhattacharya *et al.* 2022) [2]. At the same time, high temperatures towards the end of the growing season cause premature maturation, reducing biomass production and overall yield. The reproductive stage, which is especially vulnerable to heat stress, is becoming a significant limiting factor for chickpea production (Prasad *et al.*, 2012) [23]. Foliar applications of plant growth regulators and nutrients can help increase growth and productivity under both normal and late sowing conditions. Foliar application of additional nutrients plays a key role in promoting root development, enhancing nodulation, improving energy transformation, supporting metabolic processes and aiding pod formation. (Krishna and Kaleeswari, 2018) [14]. Plant growth regulators (PGRs) are known to positively influence the source-sink relationship in plants by enhancing the movement of photoassimilates. This process supports better flower formation, fruit and seed development and ultimately boosts crop productivity (Singh *et al.*, 2014) [25].

Considering the above facts, the present investigation was carried out to assess the impact of plant growth regulators and nutrients on the growth and productivity of chickpea under fluctuating environments. This study aimed to determine the best suitable sowing date and treatment as well as their interaction for a particular variety of chickpea with respect to improving the growth and yield performance of chickpea.

2. Materials and Methods

The field experiment was carried out during the *rabi* seasons of 2022-23 at the Post Graduate Research Farm, MPKV, Rahuri, Ahilyanagar, Maharashtra, India. The maximum and minimum temperatures during the experimental period were 24.1°C and 28.2°C, respectively. The experimental soil was *Vertisols* with neutral pH (8.24), electrical conductivity (EC) (0.36 dS/m²), low organic carbon content (0.68%) and available nitrogen (229.28 kg ha⁻¹). The field experiment was laid out in a split-plot design, replicated three times, to evaluate the effects of plant growth regulators and nutrients on the growth and development of chickpea (*Cicer arietinum* L.). The chickpea variety Phule Vikram was used as the test crop. The data was statistically analyzed using analysis of variance (ANOVA) as applicable to the split-plot design (Panse and Sukhatme, 1985) [22]. Pearson's Correlation Coefficient was performed to find out correlation between morphological and yield attributing traits with yield. The experiments consisted of two factors: main factor (sowing dates) and sub-factor (treatments). The sowing dates consisted of D₁ (second week of November), D₂ (first week of December) and D₃ (fourth week of December). The treatments (foliar applications) consisted of T₁ (Control), T₂ (foliar applications of Nanourea @ 0.2% at flowering and pod-filling stages), T₃ (foliar applications of Potassium Nitrate (KNO₃) @ 2% at flowering and pod-filling stages), T₄ (foliar applications of Boron @ 0.1% at flowering and pod-filling stages), T₅ (foliar applications of Kinetin @ 10 ppm at flowering and pod-filling stages), T₆ (foliar applications of CCC (Chlormequate chloride 50%) @ 30 ppm at flowering and pod-filling stages), T₇ (foliar applications of Ethrel (2-Chloroethyl phosphonic acid 39%) @ 20 ppm at flowering and pod-filling stages), and T₈ (foliar applications of NAA (α-Naphthyl Acetic Acid) @ 10

ppm at flowering and pod-filling stages). Observations were recorded on morphological traits, yield and its attributes to determine the best sowing date, treatment and their interaction with respect to study the growth and productivity of chickpea.

3. Results and Discussion

3.1. Morphological characters

The data regarding to morphological characters showed significant variations among sowing dates treatments and their interactions. The data was presented in table No. 3.1 and 3.2. According to data of sowing dates, D₁ (second week of November) was found significantly superior to increase in morphological characteristics such as plant height (66.45 cm), number of secondary branches (13.99) and leaf area (10.21 cm²). Whereas, the lowest plant height (59.64 cm), secondary branches (8.86) and leaf area (9.17 cm²) were observed in D₃ (first week of December). The increased plant height, number of secondary branches and leaf area was attributed to optimal conditions due to early sowing. Plant under late sowing condition ceases the vegetative growth to boost reproductive growth of plant. The reduction in cell division and expansion due to late sowing negatively affected morphological traits of plant. Similar findings, related to increase in morphological characteristics due to early sowing and a decrease in morphological traits due to late sowing were reported by Seth *et al.* (2020) [24] and Jadhav *et al.* (2021) [11].

According to estimated treatments, T₂ (foliar applications of Nanourea @ 0.2% at flowering and pod-filling stages) was found significantly superior to increased plant height (71.81 cm) and number of secondary branches (14.64). The number of secondary branches was at par with T₃ (foliar applications of Potassium Nitrate (KNO₃) @ 2% at flowering and pod-filling stages (13.15). The leaf area was significantly highest (10.35 cm²) in T₃ (foliar applications of Potassium Nitrate (KNO₃) @ 2% at flowering and pod-filling stages) compared to all treatments, except T₂ (foliar applications of Nanourea @ 0.2% at flowering and pod-filling stages), T₄ (foliar applications of Boron @ 0.1% at flowering and pod-filling stages), T₅ (Kinetin @ 10 ppm at flowering and pod-filling stages), and T₈ (foliar applications of NAA (α-Naphthyl Acetic Acid) @ 10 ppm at flowering and pod-filling stages). The minimum plant height (56.12 cm) and number of secondary branches (9.20) were observed in T₆ (foliar applications of CCC (Chlormequate chloride 50%) @ 30 ppm at flowering and pod-filling stages). The minimum leaf area (8.71 cm) was observed in T₁ (control). The interaction D₁T₂ (second week of November and foliar application of Nanourea @ 0.2% at flowering and pod filling stages) was found significantly superior to increased number of secondary branches at harvest, which was (17.21) as compared to other interactions. Due to easy availability of nitrogen due to foliar application of nanourea enhances metabolic processes and promotes meristematic activities, leading to higher apical growth and leaf photosynthetic area (Lakshman *et al.* 2022) [16]. Similar result related to nanourea influenced morphological characteristics of plant was given by Islam and Hussain (2023) [10] in green gram. The application of potassium nitrate supplied N and K, which may have delayed the synthesis of abscisic acid and promoted cytokinin activity, resulting in a higher the chlorophyll ratio, more leaves and leaf area (Mund *et al.*, 2024) [18].

Table 1: Influence of nutrients and plant growth regulator on morphological characters, phenological characters, yield and yield attributing characters in chickpea var. Phule Vikram under environmental fluctuations

	PH (cm)	NB	LA (cm ²)	DF 50%	DPM	NPP	100 SW	NSP	SY (kg ha ⁻¹)	BY (kg ha ⁻¹)
Sowing dates										
D ₁	66.45	13.99	10.21	50.96	106.62	95.64	21.05	1.62	2748.13	6171.81
D ₂	63.92	12.35	9.67	47.79	103.92	79.25	20.11	1.55	2563.64	6055.61
D ₃	59.64	8.86	9.17	43.88	96.54	49.93	19.32	1.48	1750.78	4992.71
S.E(m)±	0.45	0.19	0.14	0.45	0.69	0.96	0.16	0.03	14.61	51.62
CD at 5%	1.77	0.73	0.54	1.78	2.73	3.75	0.63	0.10	57.36	202.68
Treatments										
T ₁	57.11	10.94	8.71	47.00	102.44	66.80	19.09	1.50	2082.89	5502.53
T ₂	71.81	14.64	10.27	49.00	103.89	77.76	20.48	1.53	2421.60	6190.04
T ₃	65.47	13.15	10.35	47.22	100.00	85.81	21.30	1.67	2833.67	6044.89
T ₄	62.92	12.39	9.85	47.33	101.67	80.76	20.37	1.61	2590.37	5792.60
T ₅	60.89	11.21	9.94	47.33	102.22	71.92	19.83	1.56	2267.12	5616.47
T ₆	56.12	9.20	8.90	43.89	94.86	70.34	20.64	1.50	2157.63	5341.71
T ₇	64.10	10.22	9.35	51.33	108.33	68.77	19.32	1.51	2114.82	5679.95
T ₈	68.27	12.14	10.11	47.22	105.44	77.38	20.25	1.53	2365.36	5752.16
S.E(m) ±	1.03	0.41	0.21	0.58	0.69	1.12	0.20	0.03	24.33	66.64
CD at 5%	2.93	1.16	0.60	1.65	1.98	3.21	0.56	0.08	69.44	190.20
Interactions (DXT)										
S.E(m) ±	1.78	0.71	0.36	1.00	1.20	1.95	0.34	0.05	42.14	115.43
CD at 5%	NS	2.02	NS	NS	NS	5.55	NS	NS	120.27	329.43

PH- Plant height (cm), NB- Number of secondary branches, LA- Leaf area (cm²), NPP-Number of pods per plant, NSP-Number of seeds per pod, SW- 100 seed weight, SY- Seed yield (kg ha⁻¹), BY- Biological yield (kg ha⁻¹), SE- Standard error, CD- Critical difference

Table 2: Interaction effect of treatments and sowing dates on number of secondary branches, number of pods per plant, biological yield per hectare and seed yield per hectare in chickpea var. Phule Vikram under environmental fluctuations

	NB	NPP	SY (kg ha ⁻¹)	BY (kg ha ⁻¹)
D ₁ T ₁	14.03	84.17	2437.44	5688.31
D ₁ T ₂	17.21	97.10	2771.82	6936.41
D ₁ T ₃	15.99	106.58	3222.60	6482.88
D ₁ T ₄	14.77	98.35	2933.93	6304.08
D ₁ T ₅	14.07	96.18	2710.97	6098.60
D ₁ T ₆	9.80	91.75	2540.92	5762.35
D ₁ T ₇	11.20	94.58	2585.06	6028.23
D ₁ T ₈	14.85	96.43	2782.31	6073.59
D ₂ T ₁	10.47	69.33	2266.98	5711.71
D ₂ T ₂	16.46	84.40	2739.86	6456.12
D ₂ T ₃	14.42	91.40	3027.11	6342.23
D ₂ T ₄	13.59	87.36	2862.67	6131.25
D ₂ T ₅	10.96	75.80	2453.16	5874.83
D ₂ T ₆	9.49	72.27	2353.39	5574.00
D ₂ T ₇	10.89	67.84	2254.79	6160.02
D ₂ T ₈	12.55	85.61	2551.13	6194.73
D ₃ T ₁	8.33	46.89	1544.24	5107.58
D ₃ T ₂	10.27	51.78	1753.13	5177.59
D ₃ T ₃	9.05	59.44	2251.30	5309.55
D ₃ T ₄	8.80	56.56	1974.50	4942.46
D ₃ T ₅	8.60	43.78	1637.25	4875.99
D ₃ T ₆	8.30	47.00	1578.57	4688.76
D ₃ T ₇	8.57	43.89	1504.60	4851.60
D ₃ T ₈	9.00	50.11	1762.65	4988.17
SE (m)±	0.71	1.95	42.14	115.43
CD at 5%	2.02	5.55	120.27	329.43

NB- Number of secondary branches, NPP-Number of pods per plant, SY- Seed yield (kg ha⁻¹), BY- Biological yield (kg ha⁻¹), SE- Standard error, CD- Critical difference

3.2. Phenological characters

According to analysis of variance, the phenological characters showed significant variations in sowing dates and treatments but not in interactions. The data of phenological characteristics was presented in Table No. 1. Considering the sowing dates, days to 50% flowering (43.88 days) and

days to physiological maturity ((96.54 days) were significantly reduced in D₃ (first week of December). Maximum number days to 50% flowering (50.96 days) and physiological maturity (106.62 days) were observed in D₁ (second week of November). Days to flowering and physiological maturity were shortened by long photoperiod, a higher temperature regime and increased photoperiod insensitivity accelerates vegetative growth for prior flowering and maturity (Daba *et al.*, 2015) [4]. Similar findings were reported by Jadhav *et al.* (2021) [11].

According to evaluated treatments, days to 50% flowering (43.89 days) and days to physiological maturity (94.86 days) were significantly reduced in T₆ (foliar applications of CCC (Chlormequate chloride 50%) @ 30 ppm at flowering and pod filling stages). In contrast maximum days to 50% flowering (51.33 days) and days to physiological maturity (108.33 days) were observed in T₇ (foliar applications of Ethrel (2- Chloroethyl phosphonic acid 39%) @ 20 ppm at flowering and pod filling stages). The days 50% flowering and physiological maturity were reduced due to chlormequat chloride which promotes early flowering primarily by inhibiting gibberellin synthesis which promotes more compact growth which can indirectly affect flowering by altering the balance between vegetative and reproductive growth ((Iqbal *et al.*, 2017) [8]).

3.3. Yield attributing characters

The data of yield attributing characters was presented in Table 1 and 2. The data indicate that the sowing dates, treatments and interactions were significantly influenced the number of pods per plant, number of seeds per pod and 100 seed weight. According to evaluated sowing dates, sowing date D₁ (second week of November) was found significantly superior to increase number of pods per plant (95.64), number of seeds per plant (1.62) and 100 seed weight (21.05 g). In contrast, the lowest number of pods per plant (49.93), number of seeds per pod (1.48) and 100 seed weight (19.32 g) were observed in sowing date D₃ (fourth week of December). The results was might be due optimum sowing

has favorable temperature during crop growth period resulting in increased number of pods per plant, 100 seed weight and higher growth attributes. Late sowing caused poor pollen development, disrupt fertilization, pod abortion which untimely reduced number of pod per plant and 100 seed weight due to terminal heat stress (Hasanuzzaman *et al.*, 2013)^[7].

Among treatments, T₃ (foliar applications of Potassium Nitrate (KNO₃) @ 2% at flowering and at pod filling stages) was found significantly superior to increase number of pod per plant (85.81), number of seeds per pod (1.67) and 100 seed weight (21.30 g). In contrast, minimum number of pod per plant (66.80), number of seeds per pod (1.50) and 100 seed weight (19.09 g) was observed in T₁ (Control). The results were due to positive effect of potassium nitrate on pod and seed development. Potassium likely contributed to improved pod development and biomass production by boosting photosynthetic efficiency and facilitating the effective movement of nutrients to the plant's reproductive organs, resulting in greater yields (Mengal, 2001)^[17]. Parallel results regarding to foliar application of potassium nitrate @ 2% increase the number of pods per plant and 100 seed weight were reported by Kirnapure *et al.* (2020)^[13] and Mund *et al.* (2024)^[18] in chickpea.

The interaction D₁T₃ (second week of November and foliar applications of Potassium Nitrate (KNO₃) @ 2% at flowering and pod filling stages) found significantly superior to increase number of pods per plant (106.58). This result was in accordance with the investigation of Irshad *et al.* (2022)^[9].

3.4. Yield

The data regarding to yield was presented in table No. 1 and 3.2. Understanding the sowing dates, the sowing date D₁ (second week of November) was significantly superior to increase biological per hectare (6171.81 kg ha⁻¹) and seed yield per hectare (2748.13 kg ha⁻¹) while, D₃ (fourth week of December) had less biological per hectare (4992.71 kg ha⁻¹) and seed yield (1750.78 kg ha⁻¹). Elevated temperatures during the flowering and pod development stages accelerated maturity and negatively affected chickpea seed

yield (Wang *et al.*, 2006)^[29]. These findings are in accordance with Niveditha *et al.* (2022)^[20] and Vyanshvi *et al.* (2024).

Considering the treatments, treatment T₂ (foliar applications of Nanourea @ 0.2% at flowering and pod filling stages) was superior to increase biological yield (6190.04 kg ha⁻¹) while T₃ (foliar applications of Potassium Nitrate (KNO₃) @ 2% at flowering and pod filling stages) was superior to increase seed yield (2833.67 kg ha⁻¹). In contrast, Minimum biological yield (5502.53 kg ha⁻¹) and seed yield per hectare (2082.89 kg ha⁻¹) were observed in T₁ (Control). The improvement in chickpea yield under delayed sowing with potassium nitrate application could be attributed to key role of potassium in increased water uptake, enhanced osmoregulation, better stomatal movement, activation of enzymes, improved membrane stability, chlorophyll production and root development as well as nitrate role in vegetative growth (Mahmood, 2024)^[19]. Similar findings regarding to nanourea increased biological yield are corresponding with Islam and Hossain, (2023)^[10] in green gram. Similar findings regarding to potassium nitrate in improving yield are in accordance with Kirnapure *et al.* (2020)^[13] and Mund *et al.* (2024)^[18] in chickpea.

According to data of interactions, D₁T₃ (Second week of December and foliar applications of Potassium Nitrate (KNO₃) @ 2% at flowering and pod filling stage) recorded a significantly maximum seed yield per hectare (3222.60 kg ha⁻¹). While interaction D₁T₂ (Second week of December and foliar applications of Nanourea @ 2% at flowering and pod filling stage) recorded a significantly maximum biological yield per hectare (6936.41 kg ha⁻¹).

3.5. Correlation of morphological, phenological and yield attributing traits with biological and economical yield.

It's crucial to analyze the relationship between growth characteristics and their contribution to yield chickpea. Therefore the analysis was done to measure the degree of relationship of morphological, phenological and yield attributing traits with economic yield and biological yield. The data of Pearson's Correlation Coefficient presented in table 3.

Table 3: Correlation coefficient between the morphological, phenological and yield attributes characteristics with economical and biological yield

	PH (cm)	NBP	LA (cm ²)	DF 50%	DPM	NPP	100 SW (g)	BY (kg ha ⁻¹)	SY (kg ha ⁻¹)
BY (kg ha ⁻¹)	0.888831*	0.944336*	0.838717*	0.491444 ^{NS}	0.356868 ^{NS}	0.736672*	0.455777 ^{NS}	1	
SW (kg ha ⁻¹)	0.487027 ^{NS}	0.693645*	0.777013*	-0.03532 ^{NS}	-0.15652 ^{NS}	0.983398*	0.800302*	0.707798*	1

* Significant correlation at 5% = (0.482), * *Significant correlation at 1% = (0.606), PH- Plant height, NBP- Number of secondary branches per plant, LA- Leaf area, DF- Days to 50% flowering, DPM- Days to physiological maturity, SW- seed weight, BY- Biological yield, SY- Seed yield

Amongst various morphological traits, plant height, number of secondary branches and leaf area were significantly and highly positively correlated with biological yield (r=0.88*, r=0.94*, r= 0.83*) at significant correlation at 5% = (0.482). The plant height found non-significantly and positively correlated with economical yield (r=0.487^{NS}). Other morphological characters such as number of secondary branches and leaf area were found significantly and positively correlated with economical yield (r=0.693*, r=0.777*) at significant correlation at 5% = (0.482). This result shows that the plant with maximum height have more source of carbohydrate which reaches to sink and increases the biological yield of the chickpea crop. Similarly more

leaf area means more area for photosynthesis. Biabani *et al.* (2011)^[3] observed significant (p<0.01) and positive correlation with plant height (r = 0.78, p<0.01) in chick pea cultivar (Hashem).

The data of correlation coefficient between phenological traits and economic and biological yield revealed that all the phenological traits such as days to 50% flowering and days to physiological maturity were non-significantly and negatively correlated (r= -0.035^{NS}, r= -0.156^{NS}) with economic yield. Days to 50% flowering and days to physiological maturity were non-significantly and positively correlated biological yield (r= 0.491^{NS}, r=0.356^{NS}). This

result indicates that the early completion of various phenophases not beneficial for the yield of chickpea crop.

Among yield attribute traits, the number of pods per plant were significant and highly positively correlated with biological yield ($r=0.736^*$) and economical yield ($r=0.983^*$) at significant correlation at 5% = (0.482). 100 seed weight was found significantly and highly positively correlated with economical yield ($r=0.800^*$) at significant correlation at 5% = (0.482). Whereas, 100 seed weight found non-significantly and positively correlated ($r=0.455^{NS}$) with biological yield. The biological yield was also found significantly and highly positively correlated with economical yield ($r=0.707^*$) at significant correlation at 5% = (0.482).

The study of correlation in chickpea has showed that leaf area, number of secondary branches, pod number per plant, 100-seed weight and biological yield have potential contribution to increase grain yield (Noor *et al.*, 2003) [21]. This finding has close conformity with research of Kumar *et al.* (2018) [15], Usman *et al.* (2012) [27], Kaur and Bhardwaj (2019) [12] in chickpea.

4. Conclusion

Sowing date D₁ (2nd week of November) was found significantly superior to increase morphological characters, yield and yield attributing characters compare to D₂ (1st week of December) and D₃ (4th week of December) in chickpea variety Phule Vikram. D₃ (4th week of December) was found significantly reduced days to 50% flowering and physiological maturity. The foliar applications of Potassium Nitrate (KNO₃) @ 2% at flowering and pod filling stage was most effective to improving leaf area, number of pods plant⁻¹, number of seeds pod⁻¹, 100 seed weight and seed yield hectare⁻¹ which ultimately improves the growth and productivity in chickpea. The T₆ (foliar applications of CCC (Chlormequate chloride 50%) @ 30 ppm at flowering and pod filling stages) was found significantly superior to reduced days to 50% flowering and physiological maturity in chickpea variety Phule Vikram. According to Pearson's Correlation Coefficient, the morphological characters and yield attributing characteristics were significantly (significant correlation at 5%) and positively correlated with biological yield and economic yield.

5. Author's contribution

Arti Manohar Ambhore: Conducted research trial, observations, data collection, collect reviews, data analysis, interpretation of results, draft manuscript preparation and study conception and design.

Dr. S.R. Gadakh: Served as scientific advisor.

Dr. N.S. Kute: Designing experiment, reviewed manuscript and served as scientific advisor.

Dr. V.R. Awari: Critically reviewed the manuscript.

All authors reviewed the results and approved the final version of the manuscript.

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