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Impact of Exogenous Plant Growth Regulators on Physiological Traits, Tendril and Flowering in Horsegram (*Macrotyloma uniflorum* Lam) under *Kharif* Season

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ABSTRACT

Background: Horsegram is an important pulse crop grown as *Rabi* crop in Tamil Nadu. *Kharif* sown crop could not flowers may be because of more number of tendrils produced as compare to *Rabi* sown crop. Hence, an attempt was made to reduce the number of tendrils and induce flowering in horsegram during *Kharif* season by plant growth regulators.

Methods: A field experiment was conducted to study the impact of plant growth regulators *viz.,* salicylic acid (100 ppm), chlormequat chloride (CCC - 250 ppm), mepiquat chloride (250 ppm), tri iodo benzoic acid (TIBA - 200 ppm) and nitrobenzene (0.2%) on physiological traits and tendril growth associated with flowering in horsegram during *Kharif*. Different treatments were applied through foliar application at 25 Days after sowing.

Result: Among the PGRs, foliar application of TIBA registered the lowest number of tendrils (2.3) followed by CCC (3.0) compared to other treatments. Higher root length of 16.8 cm and RWC of 82.3% was found in CCC treatment. CCC also registered the highest photosynthetic rate (27.15 µmol m^{-2} s^{-1}), transpiration rate (18.06 mmol m^{-2} s^{-1}) and lowest leaf temperature (26°C) compared to other treatments. The highest soluble protein content of 13.1 mg g^{-1} was also estimated in CCC treatment followed by mepiquat chloride (12.51 mg g^{-1}). Sucrose phosphate synthase (SPS) activity did not show any significant difference between the treatments. Number of flowers buds formed per plant was zero and hence the flowering did not take place in any treatments. Among the plant growth regulators used, TIBA and CCC registered its positive action on reduced the number of tendrils and leaf temperature, but not enough to induce flowering under *Kharif* season.

Key words: CCC, Horsegram, Photosynthetic rate, SPS activity, Tendril, TIBA.

INTRODUCTION

Horsegram is widely grown pulse crop tolerant to drought and typically adapted to a wide range of soils. Among the pulses grown in India, it ranks third in area covering 17.02 lakh hectares with an annual production of 7.19 lakh tonnes. The national average productivity of horsegram is 494 kg ha^{-1} (Suthar *et al.,* 2017).

However, its photo and thermo-sensitive nature does not permit its horizontal expansion in non-traditional regions considered as a major constraint in horsegram production. *Rabi* is the only season for growing horsegram and second week of October is the optimum time of sowing in Tamil Nadu. Normally (June-July) sown horsegram does not flower may be because of more number of tendrils produced by the plant in *Kharif* season. Hence, it may be the reason why it is not grown in *Kharif* season in Tamil Nadu state.

Induction of flowering in horsegram during *kharif* season is an immense task and if achieved it will be highly valuable for increment of national production. Foliar application of TIBA produced more number of flowers per plant (Abdelkadir *et al.,* 2010) in *Jatropha curcas*. Sivakumar *et al.* (2020) reported that the application of CCC induced early flowering during *Rabi* season. Nitrobenzene is a new generation plant energizer and induces profuse flowering (Mithila *et al.,* 2012) in tomato. Pacheco *et al.* (2013) found that the foliar application of salicylic acid registered higher number of inflorescences in marigold. Exogenous application of CCC and mepiquat chloride increased the number of flowers in groundnut (Vinothini *et al.,* 2018).

Higher sucrose phosphate synthase activity was observed in CCC sprayed potato crop (Sharma *et al.,* 1998). The average number of inflorescences in plants grown at 25°C was 1.6, while no inflorescence development was observed in plants grown at 35°C was reported by Rezazadeh *et al*. (2018). Foliar application of CCC increased the relative water content in wheat under saline condition (Sharifi and Khalilzadeh, 2018). Qureshi *et al.* (2018) found that the foliar application of cycocel induced early emergence of inflorescences in chrysanthemum. Foliar

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spray with 60 mM CCC increased the relative water content, photosynthetic rate and total protein content in pearl millet under drought stress condition (Santosh Kumari, 2017).

Production of more number of tendrils might be a major constraint and it acts as a sink and affects source-sink relationship ultimately flowering. Secondo and Reddy (2018) found that the greater proportion of photo-assimilates diverted for vegetative growth rather than reproductive parts leads to more plant height. Above statement clearly indicated that diversion of photosynthates to tendril which is vegetative part of horsegram can arrest the flowering. Based on this background, a study was undertaken for the induction of flowering in horsegram under *Kharif* season using plant growth regulators. This was the first attempt made to induce flowering in horsegram during *Kharif* season.

MATERIALS AND METHODS

A field experiment was conducted at Regional Research Station, Paiyur, Krishnagiri District, Tamil Nadu Agricultural University during 2019 to 2021 (Two years) under rainfed condition. Horsegram variety Paiyur 2 seeds were sown with the spacing of 30 × 10 cm during first week of July with the plot size of 2 m × 1.5 m. Plant growth regulators include salicylic acid (SA - 100 ppm), chlormequat chloride (CCC - 250 ppm), mepiquat chloride (MC - 250 ppm), tri iodo benzoic acid (TIBA - 200 ppm) and nitrobenzene (NB - 0.2%) were applied as foliar spray at 25 days after sowing along with control maintained with water spray. The experiment was carried out in randomized block design with three replications.

To measure the root length, the plants were uprooted and wash it carefully to ensure minimum damage and the length from the cotyledonary node to the root tip was measured and expressed in cm. Number of tendrils per plant was counted manually in each replications and average of five plants per treatment was taken. The relative water content (RWC) was estimated according to Barrs and Weatherley (1962) and calculated it by using following formula and expressed as per cent.

$$RWC = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Measurement of gas exchange parameters was performed by using an instrument Portable Photosynthesis System (PPS) (Model LC*pro*-SD., ADC BioScientific Ltd., Hoddesdon, UK) equipped with a halogen lamp (6400-02B LED) positioned on the cuvette. Third leaf from the top was used for the measurement and replicated thrice. Leaf was inserted in 3 cm^2 leaf chamber and PPFD at 1500 µmol photons m^{-2} s^{-1} and relative humidity (50-55%) were set. The photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol CO_2 m^{-2} s^{-1}) and leaf temperature (°C) were measured between 9 am to 11.30 am and the value was expressed with respective units. Soluble protein content of leaf was estimated as per the method of Lowry *et al*. (1951) and expressed as mg g^{-1} fresh weight. Sucrose phosphate synthase activity was determined as described by Pavlinova *et al.* (2002). Sucrose content was estimated using anthrone reagent as the method modified by Ashwell (1957). Absorbance was measured at 630 nm and the activity was expressed in mg sucrose mg $protein^{-1}$ h^{-1}. Number of flower buds formed per plant was counted manually and average of five plants was taken. The data on various parameters were analyzed statistically as per the procedure suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Growth traits

Estimation of plant root length is considered as an important parameter for the development of the plant. In the present study, the highest root length of 16.8 cm was recorded with the treatment of CCC followed by TIBA (15.9 cm) while the lowest length of 13.7 cm registered in absolute control (Fig 1). Foliar application of mepiquat chloride (15.6 cm) and salicylic acid (15.2 cm) also showed positive impact on root length. Sivakumar and Nandhitha (2017) reported that the root length was increased by the application of salicylic acid in mung bean under salt stress. The root length improves water and nutrient absorption from soil and helps plant growth and development. The increased in root length was up to 22.6% with CCC application. The increase in root length with CCC might be due to the diversion of photo-assimilates to root growth instead of tendril growth by its growth retardant nature. Jimenez *et al.* (2018) found that enhancement of floral buds was observed with the plant having higher root volume, increased concentration of sugars and amino acids in pepper. The study of Anosheh *et al.* (2016) showed that the improvement in root growth may be because of increased IAA contentment with the CCC treatment.

Present study corroborated with the earlier investigation. Talebi *et al.* (2014) reported that the increased root dry weight helps to produce higher flower count in *Gajania* might be due to absorption of water and nutrients effectively from the soil.

Horsegram leaf has tendrils that permit climbing plants together to their neighbours. Formation of tendrils indicates that the vegetative growth of the plant is continues. Even though tendril is a vegetative part, it is not useful for photosynthesis and flowering, however it utilizes photosynthates for its growth ultimately inhibit flowering. Hence, decrease the number of tendrils is a pre requisite for the induction of flowering.

In the present study, the highest number of tendrils per plant was recorded in control (6.7) while the lowest number of tendrils registered by the foliar spray of TIBA (2.3) followed by CCC which recorded 3 numbers of tendrils per plant (Fig 2). It clearly indicated that TIBA reduced the number of tendrils might be due to arresting the apical dominance by its anti-auxin role and CCC through its anti-gibberellin activity. Mansuroglu *et al.* (2009) reported that most plant growth retardants inhibit the growth by arresting the active gibberellins synthesis and reduce unwanted shoot elongation. Foliar application of CCC reduced the number

of tendrils in horsegram during *Rabi* season was reported by Sivakumar *et al.* (2020). Present study corroborated with earlier findings.

Gas exchange parameters

Exogenous application of CCC increased the photosynthetic rate and transpiration rate in horsegram under *kharif* season. CCC recorded higher photosynthetic rate of 27.15 µmol m^{-2} s^{-1} followed by salicylic acid (25.52 µmol m^{-2} s^{-1}) and the control plant showed lowest rate of photosynthesis (Table 1). The per cent increase in photosynthesis was 11% more than control with CCC treatment. The increased in transpiration might be the possible reason of increase in photosynthesis with the treatment of CCC.

Wang *et al.* (2010) found that the application of CCC prevents chlorophyll destruction in potato and facilitate maintenance of photosynthetic rate. Foliar application of 1% TNAU Horsegram Wonder (Specific foliar formulation for Horsegram to increase the grain yield) increased the photosynthetic rate might be due to CCC as major component of the formulation (Sivakumar *et al.,* 2020). This result corroborated with the present study.

The lowest transpiration rate was recorded in water sprayed control (12.68 mmol m^{-2} s^{-1}), while foliar spray of CCC registered the highest transpiration rate of 18.06 mmol m^{-2} s^{-1}. Maintenance of transpiration rate is an essential to maintain the photosynthetic rate and leaf temperature. Among the treatments, CCC increased the transpiration rate up to 42.4% compared to control. This condition is attributed to reduce leaf temperature which helps the plants to tolerate high temperature.

Leaf temperature is an important physiological trait for plant growth and flower induction. Foliar spray of CCC reduced the leaf temperature compared to unsprayed one. Water sprayed control registered higher leaf temperature of 27.6°C. However, the lowest leaf temperature of 26°C was found in CCC spray which is at on par with salicylic acid (26.6°C). The increased transpiration rate directly lowers the leaf temperature contributed positively to the plant health. In the present study, leaf temperature was reduced

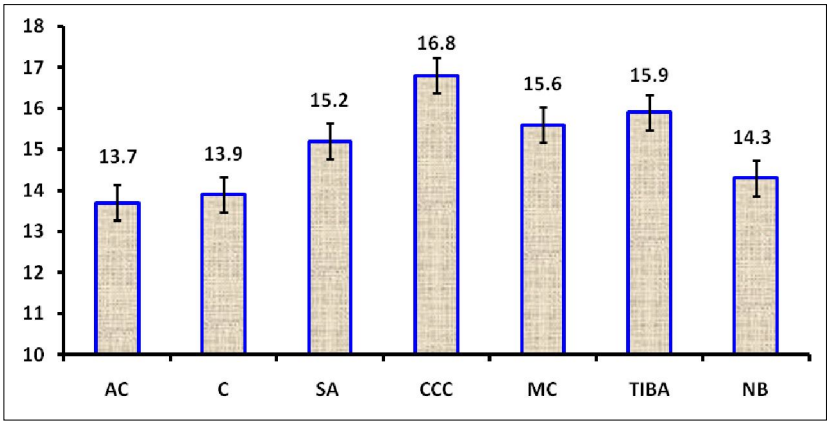

Fig 1: Effect of plant growth regulators on root length (cm) in *Kharif* horsegram.
AC- Absolute control, C- Control, SA- Salicylic acid (100 ppm), CCC- Chlormequat chloride (250 ppm), MC- Mepiquat chloride (250 ppm), TIBA- Tri iodo benzoic acid (200 ppm), NB- Nitrobenzene (0.2%).

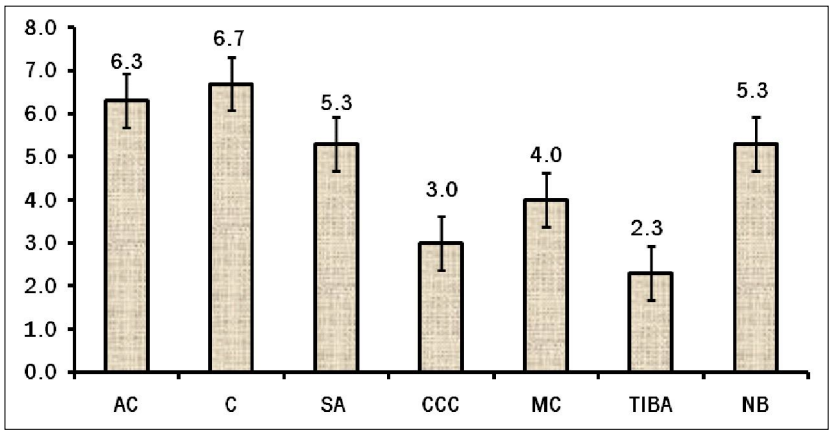

Fig 2: Effect of plant growth regulators on tendril number in *Kharif* horsegram.
AC- Absolute control, C- Control, SA- Salicylic acid (100 ppm), CCC- Chlormequat chloride (250 ppm), MC- Mepiquat chloride (250 ppm), TIBA- Tri iodo benzoic acid (200 ppm), NB- Nitrobenzene (0.2%).

up to 1.6°C by the application of CCC compared to control. The reduced leaf temperature by the application of CCC might be due to increased transpiration rate which cools the plant. Anosheh *et al.* (2012) reported that the canopy temperature reduced by the application of CCC in wheat may be due to improving stomatal regulation.

Relative water content (RWC)

It is an appropriate physiological trait which measures plant water status and also photosynthesis. CCC showed positive impact on RWC compared to control (Table 1). Application of CCC maintained higher RWC, even though it registered higher transpiration rate might be due to longer root which facilitates absorption of more water from the deeper layer. Similarly control plants showed lower transpiration and RWC might be due to poor water absorption through shorter root length. Among the treatments, CCC sprayed plants registered highest RWC of 82.3% followed by mepiquat chloride (78.6%). Unsprayed absolute control recorded lowest RWC of 73.8% followed by water spray control (74.1%).

In the present study, application of CCC increased the RWC up to 11.5% compared to absolute control. Jiriaie and Sajedii (2012) reported that the CCC application increased RWC in wheat. The present study agreed with the earlier investigation. Even though CCC application increased the transpiration rate, the RWC of the plant could be maintained by the increment of root length to absorb water from the deep soil. In the case of flowering, no flower buds formed in any treatments. Number of flower buds counted was zero in all the treatments and transformed value was presented in the Table 1. Hence, there was no difference observed among the treatments in number of flower buds formed.

SPS activity

Sucrose phosphate synthase (SPS) is the plant enzyme which plays a major role in sucrose biosynthesis and sustains the assimilatory carbon flux from source to developing sink (Isopp *et al.,* 2000). Chen *et al.* (2005) found that SPS enzyme involved in the partitioning of photo-assimilates and regulating the partitioning of carbon between starch production and carbohydrate accumulation in many physiological and developmental processes. Baxter *et al.* (2003) reported that the increased rates of SPS activity have accelerated flower development and profound impact on flowering.

Table 1: Impact of plant growth regulators on gas exchange parameters, RWC, SPS activity and number of flowers in *Kharif* horsegram.

Treatments	Photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$)	Transpiration rate ($mmol\ m^{-2}\ s^{-1}$)	Leaf temperature (°C)	RWC (%)	SPS activity (mg sucrose mg $protein^{-1}\ h^{-1}$)	Number of flowers per plant*
Absolute control	24.46	12.78	27.4	73.8	1.21	0.432
Control	24.64	12.68	27.6	74.1	1.21	0.432
Salicylic acid	25.52	15.30	26.6	77.5	1.25	0.432
CCC	27.15	18.06	26.0	82.3	1.31	0.432
Mepiquat chloride	24.95	14.91	26.8	78.6	1.29	0.432
TIBA	24.77	14.92	26.9	76.9	1.34	0.432
Nitro benzene	24.84	13.15	27.2	74.7	1.31	0.432
SEd	0.682	0.375	0.302	1.98	0.088	0.0092
CD (P=0.05)	1.377	0.809	0.611	4.14	0.179	0.0217

*Transformed data.

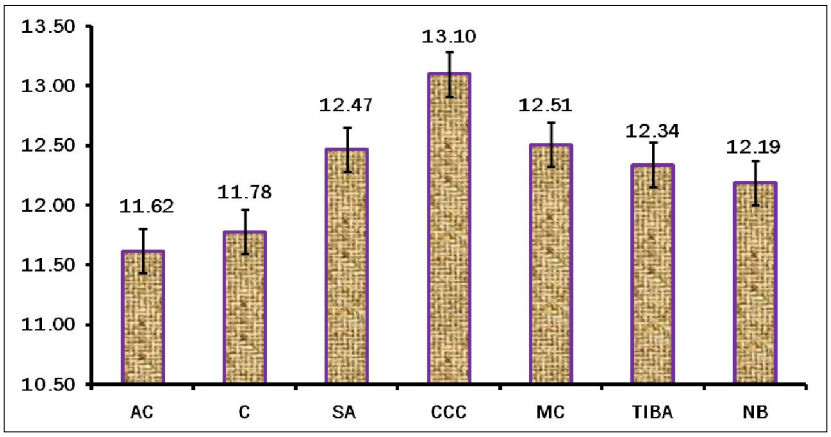

Fig 3: Effect of plant growth regulators on soluble protein content in *Kharif* horsegram.
AC- Absolute control, C- Control, SA- Salicylic acid (100 ppm), CCC- Chlormequat chloride (250 ppm), MC- Mepiquat chloride (250 ppm), TIBA- Tri iodo benzoic acid (200 ppm), NB- Nitrobenzene (0.2%).

Application of TIBA showed higher SPS activity (1.34) followed by CCC (1.31) compared to control (1.21). However, exogenous application of plant growth regulators did not show any significant difference in the case of SPS activity. This may be one of the reasons for absence of flowering in horsegram under *Kharif* season.

Soluble protein

Rubisco enzyme occupied more than 50 per cent of the soluble proteins in crop plants was reported by Myat *et al.* (2014). Hence, the soluble protein content is an indirect index for assessing photosynthetic efficiency of crop plants. Among the treatments, foliar application of CCC registered higher soluble protein content of 13.10 mg g^{-1} followed by mepiquat chloride (12.51 mg g^{-1}) and salicylic acid (12.47 mg g^{-1}) while lower was recorded in absolute control (11.62 mg g^{-1}).

An increment of 12.7% soluble protein was observed in the present study by the application of CCC (Fig 3). Bhagure and Tamble (2013) reported that the CCC has the ability to promoting the synthesis of soluble protein. An increment of 15.4% soluble protein content was observed in horsegram by the application of TNAU Horsegram Wonder which contains CCC as main component (Sivakumar *et al.,* 2020). The present study corroborated with the earlier findings.

CONCLUSION

Exogenous application of CCC (250 ppm) showed some positive initiation on the reduced number of tendrils, enhancement of RWC, soluble protein and photosynthetic rate. However, it does not show any significant impact on SPS activity. Hence, it is not enough to produce flowers even in the favorable physiological traits achieved except SPS activity. Further study is required on gene profiling under *Kharif* season to achieve the target.

REFERENCES

Abdelkadir, H.A., Jager, A.K., Johnson, S.D. and Staden, J.V. (2010). Influence of plant growth regulators on flowering, fruiting, seed oil content and oil quality of *Jatropha curcas*. South African Journal of Botany. 76(3): 440-446

Anosheh, H.P., Emam, Y., Ashraf, M. and Foolad, M.R. (2012). Exogenous application of salicylic acid and chlormequat chloride alleviates negative effects of drought stress in wheat. Advanced Studies in Biology. 4(11): 501-520.

Anosheh, P., Emam, Y. and Khaliq, A. (2016). Response of cereals to cycocel application. Iran Agricultural Research. 35(1): 1-12.

Ashwell, G. (1957). Colorimetric Analysis of Sugars. In: Methods in Enzymology, [Colowick, S.P. and Kaplan, N.O. (eds.)], III, 73-105, Academic Press, New York.

Barrs, H.D. and Weatherley, P.E. (1962). A re-examination of relative turgidity for estimating water deficits in leaves. Australian Journal of Biological Sciences. 15: 413-428.

Baxter, C.J., Foyer, C.H., Turner, J., Rolfe, S.A. and Quick, W.P. (2003). Elevated sucrose-phosphate synthase activity in transgenic tobacco sustains photosynthesis in older leaves and alters development. Journal of Experimental Botany. 54: 1813-1820

Bhagure, Y.L. and Tamble, T.B. (2013). Effect of seed soaking and foliar sprays of plant growth regulators on germination, growth and yield of okra [*Abelmoschus esculentus* (L.) Moench var]. Parbhani Kranti. The Asian Journal of Horticulture. 8(2): 399-402.

Chen, S., Hajirezaei, M. and Bornke, F. (2005). Differential expression of sucrose-phosphate synthase isoenzymes in tobacco reflects their functional specialization during dark-governed starch mobilization in source leaves. Plant Physiology. 139(3): 1163-1174.

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedures for Agricultural Research. (2nd Ed.) John Wiley and sons, New York, USA.

Isopp, H., Frehner, M., Long, S.P. and Nosberger, J. (2000). Sucrose phosphate synthase responds differently to source-sink relations and photosynthetic rates: *Lollium perenne* L. growing at elevated CO_2 in the field. Plant, Cell and Environment. 23: 597-607.

Jimenez, A.G., Tellez, L.I.T., Sanchez, D.G. and Merino, F.C.G. (2018). Vanadium stimulates pepper plant growth and flowering, increases concentrations of amino acids, sugars and chlorophylls and modifies nutrient concentrations. PLoS ONE. 13(8): e0201908

Jiriaie, M. and Sajedii, N.A. (2012). Effect of plant growth regulators on agro physiological traits of wheat [*Triticum aestivum* (L.) var. Shahriar] under water deficit stress. Research on Crops. 13 (1): 37-45.

Lowry, O.H., Brought, N.T.R., Farr, L.A. and Randall, R.J. (1951). Protein measurement with folin phenol reagent. Journal of Biological Chemistry. 193: 265-275.

Mansuroglu, S., Karaguzel, O., Ortacesme, V. and Sayan, M.S. (2009). Effect of paclobutrazol on flowering, leaf and flower colour of Consolida orientalis. Pakistan Journal of Botany. 41: 2323-2332.

Mithila, D., Sajal, R. and Imamul Huq, S.M. (2012). Effects of nitrobenzene on growth of tomato plants and accumulation of Arsenic. Bangladesh Journal of Scientific Research. 25: 43-52.

Myat, T., Lin, A., Occhialini, P., John Andralojc, A.J., Martin, M., Parry, M. and Hanso, R. (2014). A faster Rubisco with potential to increase photosynthesis in crops. Nature. 513: 547-550.

Pacheco, A.C., Cabral, C.S., Fermino, E.S.S. and Aleman, C.C. (2013). Salicylic acid-induced changes to growth, flowering and flavonoids production in marigold plants. Journal of Medicinal Plant Research. 7(42): 3158-3163.

Pavlinova, O.A., Balakhontsev, E.N., Prasolova, M.F. and Turkina, M.V. (2002). Sucrose phosphate synthase, sucrose synthase and invertase in sugar beet leaves. Russian Journal of Plant Physiology. 49: 68-73.

Qureshi, I.A., Gulzar, S., Rashid, A., Rehman, R. and Inayatullah, T. (2018). Effect of growth retardants on the growth and flowering of *Chrysanthemum morifolium* cv. Flirt. Indian Journal of Agricultural Research. 52(3): 319-322.

Rezazadeh, A., Harkess, R.L. and Telmadarrehei, T. (2018). The effect of light intensity and temperature on flowering and morphology of potted Red Firespike. Horticulturae. 36(4): doi: 10.3390/horticulturae4040036.

Santosh Kumari (2017). Effect of kinetin (6-FAP) and cycocel (CCC) on growth, metabolism and cellular organelles in Pearl Millet (*Pennisetum glaucum*) under water stress. International Journal of Current Microbiology and Applied Sciences. 6(8): 2711-2719.

Secondo, A.S.P. and Reddy, Y.A.N. (2018). Plant growth retardants improve sink strength and yield of sunflower. International Journal of Current Microbiology and Applied Sciences. 7(10): 111-119.

Sharifi, R.S. and Khalilzadeh, R. (2018). Effects of cycocel on growth, some physiological traits and yield of wheat (*Triticum aestivum* L.) under salt stress. Journal of Plant Physiology and Breeding. 8(1): 11-23.

Sharma, N., Kaur, N. and Gupta, A.K. (1998). Effect of chloro choline chloride sprays on the carbohydrate composition and activities of sucrose metabolising enzymes in potato (*Solanum tuberosum* L.). Plant Growth Regulation. 26: 97-103.

Sivakumar, R. and Nandhitha. G.K. (2017). Impact of PGRS and nutrients pre-soaking on seed germination and seedling characters of mung bean under salt stress. Legume Research - An International Journal. 40(1): 125-131.

Sivakumar, R., Vijayakumar, M. and Tamilselvan, N. (2020). Impact of foliar spray of PGR nutrient consortium on growth, photosynthesis and yield of horsegram (*Macrotyloma uniflorum* Lam) under rainfed condition. Legume Research - An International Journal. DOI: 10.18805/LR-4413.

Suthar, R., Patel, P.H., Kumar, A. and Urmila. (2017). Effect of horsegram (*Macrotyloma uniflorum* Lam. Verdec) varieties and different row spacing on yield attributes and yield. Life Sciences International Research Journal. 4: 1-6.

Talebi, M., Hadavi, E. And Jaafari, N. (2014). Foliar sprays of citric acid and malic acid modify growth, flowering and root to shoot ratio of Gazania (*Gazania rigens* L.): A comparative analysis by ANOVA and structural equations modelling. Advances in Agriculture. http://dx.doi.org/10.1155/2014/147278.

Vinothini, N., Vijayan, R. and Umarani, R. (2018). Impact of foliar application of plant growth regulators on seed filling and seed multiplication rate in groundnut (*Arachis hypogaea* L.). International Journal of Chemical Studies. 6(5): 2186-2189.

Wang, H., Xiao, L., Tong, J. and Liu, F. (2010). Foliar application of chloro choline chloride improves leaf mineral nutrition, antioxidant enzyme activity and tuber yield of potato (*Solanum tuberosum* L.). Scientia Horticulturae. 125: 521-523.