

Effect of different doses of silver nitrate on flowering behavior and seed development in Cucumber (*Cucumis sativus* L.)

Rajan P. Mishra *¹, Gyan singh¹, Shlok Bhalinge¹, Sandeep Kumar² Rohidas A Waghmare¹, Ashok Kumar³

1* Senior Breeder Cucurbits at Namdeo umaji Agritech India Pvt. LTD. Division of research and development
Uruli kanchan Pune 412202.

2- Bhagwant University Ajmer Rajasthan

3- Institute of agriculture science BundelKhand University Jhansi Uttar Pradesh

Corresponding Author's Email- rajanmishrask@gmail.com

1. INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the potential greenhouse vegetable crops (*Cucumis sativus* L., $2n=2x=14$), and the availability of parthenocarpic cultivars has actually revolutionized greenhouse cucumber production in India. Parthenocarpy is a much-desired trait in cucumber production as it minimizes yield irregularity, enhances total yield, and makes the production possible under suboptimal environmental conditions (Dhatt and Kaur, 2016). So the introduction of parthenocarpic cucumber varieties enabled the farmers to grow the cucumber inside the greenhouse without any pollinators to get a higher income.

It is thought to be one of the oldest vegetable crops, being grown for at least five thousand years. It is the fourth most important vegetable crop after tomato, cabbage, and onion in Asia. The fruits are used for the preparation of cosmetic items like soap and cream and in many other ways (Dhiman and Parkash, 2005). The crop is of Asian origin and the progenitor may be closely related to its wild relative *Cucumis sativus* var. *hardwickii*, first found in the Himalayan Mountains (foothills of Nepal) and used by native peoples of Northern India as a laxative (Deakin et al., 1971). India being the primary center of origin, it has accumulated a wide range of variability providing a good extent for improvement in yield and other characteristics of cucumber through selection pattern.

Abstract - This study was conducted to investigate the effect of deferent PPM doses of Silver Nitrate ($AgNO_3$) on flowering behavior, seed development, and pollen quantity in induced flower in cucumber genotype. In order to investigate the applications, the effect of $AgNO_3$ on male flower generative characteristics of the planting period (Summer-2021) was identified, and the result was statistically evaluated. In this research result was obtained is that $AgNO_3$ has led to the formation of male flowers, Moderate concentration dose of $AgNO_3$ performed well and produced enough male flowers as well as good pollen quantity while high concentration doses are phytotoxic and low concentration doses unable to induce staminate flower, controlled has not to produce male flower et all.

In the present study, it was intended to exhibit the effect of different doses of $AgNO_3$ applications on the gender formation of the cucumber genotype, boost male flower formation to identify the level of silver nitrate application that would best boost the formation of male flowers with good pollen quantity and develop practical recommendations on these issues.

Key Words: $AgNO_3$, Sex expression, Parthinocarpy, Seed development

Cucumber is a highly cross-pollinated crop so, it's very difficult to maintain purity in Germplasm but in Parthenocarpic cucumber there is no pollination evolving in fruit formation, so there is no problem in commercial production of such type cucumber. Parthenocarpic cucumber has always higher yield than normal monoecious cucumber, while common cucumber always required less expenditure than Parthenocarpic cucumber. Thus breeding in parthenocarpic cucumber is important and demandable, it shows a wide range of variability in existing germplasm. Challenging traits as per market demand and commercial points of view such as uniformity, fruit size, shape, color, quality, and yield are important traits.

The parthenocarpic genotypes bear all-female flowers in every node producing seedless fruits depending on pc genes in the background (Pike and Peterson, 1969) sometimes multipistilate (more than one female flower) flower present on a single node is also recorded, multipistilate nature of plant enhance yield/fruit productivity in cucumber. Therefore, breeding activities are required to induce male flowers. Many chemical and plant hormones (AgNO₃, Sodium thiosulphate & GA) are used for male inducing in parthenocarpic and gynocious germplasm maintenance and breeding.

In almost all commercial cucumber growing (both field and Greenhouse) in the world, the parthenocarpic and monoecious kinds are used. With this type, efficiency is directly proportional to the number of female flowers. However, factors like light intensity, light duration, and temperature can affect ecological factors on the generative structure of cucumber. For example, while the high temperature and long days in the cultivation period increase the number of Male flowers of cucumber, they lead to the reduction of the number of the female flower, and hence cause the degradation of efficiency (Cantliffi, 1981).

Cucumber has three types of flowering habit "gynoecious" (GY) which produces only female flowers, "predominantly gynoecious" (PDG) or

"predominantly female" (PDF) which bears mainly female flowers along with some male flowers and "monoecious" which produces both male and female flowers somewhat in equal proportions (Mehdi et al., 2012). Growth regulators have tremendous effects on sex expression and flowering in various cucurbits leading to either suppression of male flowers or an increase in the number of female flowers without imposing any deleterious effects on the environment and human health. Exogenous application of plant growth regulators can alter the sex ratio and sequence if applied at the 2-4 leaf stage, which is the critical stage at which the suppression or promotion of either sex is possible (Kumar et al., 2011).

Staminate flower induction is necessary for the production of F1 hybrid in gynocious Parthenocarpic cucumber. Parthenocarpic sex expression has been responsible for the exploitation of hybrid vigor in cucumber which has attained a high degree of perfection (Golabadi et al., 2015). The improvement of any crop plant which is the ultimate objective of the plant breeder worldwide is not a simple task. Somewhere breeders fail due to a lack of appropriate knowledge of formulation of such chemical or plant growth hormone while they using full effort for breeding.

The basic idea behind this study was to develop an effective dose formulation technique of Silver Nitrate (AgNO₃) for maintaining the Parthenocarpic line under poly-house so that the Parthenocarpic line could be used successfully as a parent in deferent hybridization program of cucumber. Such a study aims to develop the basis for any future hybridization program for improvement and standardization of the local cultivars. So the present study was formulated to evaluate the effect of AgNO₃ to identify the best dose for induction of staminate flower (with good quantity and high-quality pollen in Parthenocarpic genotypes).

2. MATERIALS AND METHODS

2.1. Material and Experimental site

The experimental material for the present investigation was taken from the Cucumber Line development program (LDP) of Namdeo Umaji Agritech (I) Pvt. Ltd. Research and Development Division (R&D). The experiment was conducted at the main R&D station Uruli Kanchan Pune Block 'E'.

Sr.	Line name/Code	Source
1	NUCUSU21-01	Namdeo Umaji Agritech (I) Pvt. Ltd.
2	NUCUSU21-02	Namdeo Umaji Agritech (I) Pvt. Ltd.
3	NUCUSU21-03	Namdeo Umaji Agritech (I) Pvt. Ltd.
4	NUCUSU21-04	Namdeo Umaji Agritech (I) Pvt. Ltd.
5	NUCUSU21-05	Namdeo Umaji Agritech (I) Pvt. Ltd.
6	NUCUSU21-06	Namdeo Umaji Agritech (I) Pvt. Ltd.
7	NUCUSU21-07	Namdeo Umaji Agritech (I) Pvt. Ltd.
8	NUCUSU21-08	Namdeo Umaji Agritech (I) Pvt. Ltd.

Table:-1 List of experimental Material

2.2. Experimental layout details

Design- RBD (Two way factorial analysis)

Replication-3

Spacing- (Row to Row) X (Plant to Plant) – 120 X 60 cm

Plot Size- 3.5 M X 3.8 M

Population – 18 plants per genotype (6 Plant per replication 6X3=18)

The silver nitrate application was performed by the method of hand spraying on the growth tips of plants and stem nodes of the plants, doses were 0 (controlled or blank), 200ppm, 400ppm, 600ppm, 800ppm, and 1000ppm. The AgNO₃ application was conducted evening time after 17.30 pm when sunlight is almost deemed (just before sunset) to avoid the sunburn of the plants. The irrigation was conducted in the form of drip irrigation and the necessary fertilization was applied in the form of fertigation.

2.3. Steps of solution preparation

Silver nitrate (AgNO₃) chemical from EMPARTA-ACS chemical producing company used for spray. The only fresh solution is prepared for use (30 minutes before using). Double distilled water is used for dissolving silver nitrate (AgNO₃). One liter Black color painted hand sprayer used for solution spray (Fig.-3)

Controlled (0 Mg Water) ,200ppm (0.315 Mg), 400ppm (0.630Mg), 600ppm (0.948Mg), 800ppm (1.26g) and 1000ppm (1.57g) of silver nitrate was prepared in distilled water and final volume was made one liter in each case.

2.4. Statistical analysis

The response of different doses of AgNO₃ for the induction of male flowers in gynoecious lines was analyzed in a randomized block design (RBD). The statistical analysis was carried out for each observed character under the study using two way factorial analysis in MS-Excel and OPSTAT 16.0 software as per the designs of the experiments.

3. Result and discussion

Yield is an important trait to accept the variety by the farmer, especially for a protected environment. Sex expression is a characteristic that determines the yield and yield potential of a different cucumber variety. Use of AgNO₃, which is involved in the maintenance of gynoecious traits in parthenocarpic genotype and leading to modification in the sex form. The spray of different concentrations dose of AgNO₃ was applied at the 3rd leaf stage (Fig.3) at 3 days interval till the 12- to 15 leaf stage. Male flowers were induced after the 4th spray of 1000 PPM, 6th spray of 800 PPM 10th spray of 600ppm 12th spray of 400 PPM, while flowers were not induced at 200 PPM and controlled. The subsequent effect of different doses of AgNO₃ is shown in **Fig.4**



Fig:-1 Genotype



Fig. 4- 1- Male Flower expression, 2&3- Female flower expression in plant



Fig. 3- i & ii- used AgNO₃ chemical, iii & iv – Plant stage for AgNO₃

The observations recorded on the mean number of male flowers induce through varied concentrations of AgNO₃ showed significant variation (**Table 2**). The mean comparisons showed that the AgNO₃ dose had a significant effect on the number of staminate flower induction whereas no male flower has been noticed in the control treatment (Distilled water). The mean number of male flowers induced through AgNO₃ at 800ppm was more and found significantly higher than other concentrations 1000ppm was also found to be significant due to

higher concentration of AgNO₃ but the phytotoxic effect was also noticed in all varieties whereas 600ppm 400ppm and 200ppm or non-significant while Controlled unable to produce Male flower, so 800ppm AgNO₃ producing high no of male flower among all concentration. 800ppm dose of Silver nitrate not only produces a high number of flowers but also no phytotoxic effect than 1000ppm all treatment e.g. - 600ppm and 400ppm. 800ppm and 1000ppm Concentrations of AgNO₃ had a significant effect on the induction of male flowers (**Fig.2**)

Maximum number of male flower induced through AgNO₃ was recorded in 800 ppm NUCUSU21-04 (338), NUCUSU21-08 (331), NUCUSU21-05 (278), NUCUSU21-07 (276), NUCUSU21-06 (274), NUCUSU21-02 (271), NUCUSU21-01 (265), NUCUSU21-03 (256) & 1000 ppm NUCUSU21-08 (235), NUCUSU21-04 (213), NUCUSU21-03 (203), NUCUSU21-05 (197), NUCUSU21-06 (196), NUCUSU21-07 (178), NUCUSU21-02 (175), NUCUSU21-01 (85) and no male flower was found in controlled conditions (Distilled water). Similarly in other concentration show, mean number of male flower induced through AgNO₃ at 600ppm NUCUSU21-04 (183), NUCUSU21-03 (145), NUCUSU21-08 (115), NUCUSU21-02 (40) 400 ppm NUCUSU21-08 (43), NUCUSU21-02 & NUCUSU21-03 (34), NUCUSU21-01 (33), NUCUSU21-05 (27), NUCUSU21-07 (17), NUCUSU21-04 (16), NUCUSU21-06 (13) & 200 ppm NUCUSU21-04 (9),

NUCUSU21-05 (104), NUCUSU21-06 (73), NUCUSU21-01 (69), NUCUSU21-07 (66), NUCUSU21-02 (8), NUCUSU21-03, NUCUSU21-05 & NUCUSU21-01 (7), NUCUSU21-08 (6), NUCUSU21-06 & NUCUSU21-07 (5). (Table: - 4)

3.1. Silver Nitrate (AgNO₃) dose-effect on plant Morphology in Cucumber

Silver Nitrate (AgNO₃) Dose effect badly on plant Morphology like mishandling, wrong time spray, or contaminated solutions. High concentration of

AgNO₃ stuck plant growth resulting in plant show leaf curling, brown spot on leaf and stem cracking may occur. So this study indicates that a higher concentration of silver nitrate is phytotoxic Hirayama and Alonso (2000), Law et al., (2002), Stankovic and Prodanovic (2002), Hallidri (2004), Golabadi et al., (2015), Meena (2015)

ANOVA						
SOURCE	DF	SS	MS	F CAL	RESULT	P value
REP	2	11039.94	5519.97	1.097081	NS	0.33447
Variety	7	37314.96	5330.709	1.059466	NS	0.38823
Concentration	39	2892261	74160.55	14.73923	**	0
Variety X Concentration	273	3420361	12528.79	2.490068	**	0
ERROR	638	3210101	5031.506			
TOTAL	959	9571078				

Table: 2- Analysis of variance

MEAN TABLES									
Variety	MEAN	Concentration	MEAN	A/B	200 PPM	400 PPM	600 PPM	800 PPM	1000 PPM
NUCUSU21-01	16.34167	200 PPM	5.916667	NUCUSU21-01	8	32.66667	71	275.3333	266.6667
NUCUSU21-02	25.13333	400 PPM	17.83333	NUCUSU21-02	8.666667	33.33333	39.33333	650	274
NUCUSU21-03	36.625	600 PPM	84.04167	NUCUSU21-03	11.66667	32.66667	263.3333	900	257.3333
NUCUSU21-04	20.06667	800 PPM	293.25	NUCUSU21-04	11.66667	17.33333	191	153.3333	429.3333
NUCUSU21-05	26.475	1000 PPM	222.1667	NUCUSU21-05	7.333333	26.66667	107.6667	367.3333	550
NUCUSU21-06	15.79167			NUCUSU21-06	5.333333	13.66667	136	200.6667	276
NUCUSU21-07	22.73333			NUCUSU21-07	5.333333	16.66667	65	516.6667	305.6667
NUCUSU21-08	25.55			NUCUSU21-08	6	43.66667	146.6667	403.3333	422.3333

Table: 3- Mean performance of analysis of variance

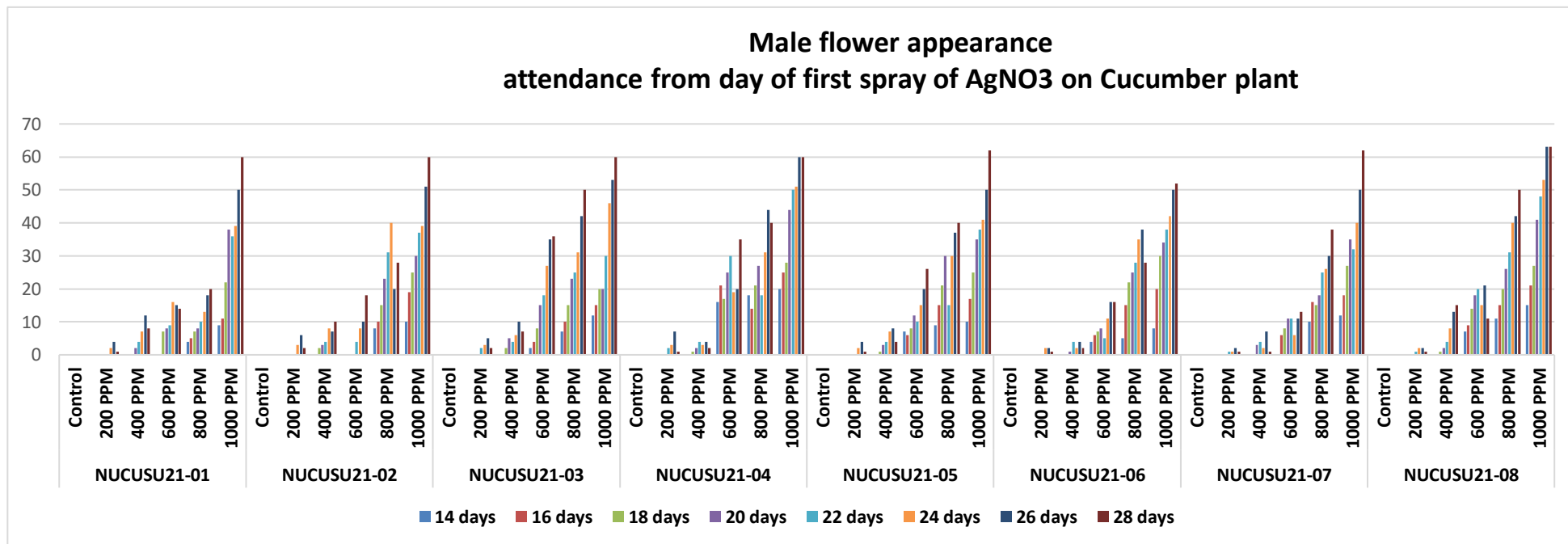


Fig. - 2 Comparison of Male flower appearance between different days and number of spray

Genotype	Control		200 PPM		400 PPM		600 PPM		800 PPM		1000 PPM	
	Flower	Seed/Fruit	Flower	Seed/Fruit	Flower	Seed/Fruit	Flower	Seed/Fruit	Flower	Seed/Fruit	Flower	Seed/Fruit
NUCUSU21-01	0	0	7	10	33	30	69	55	265	63	85	50
NUCUSU21-02	0	0	8	22.2	34	36	40	48	271	80	175	75
NUCUSU21-03	0	0	7	18	34	29.6	145	60	256	71	203	75
NUCUSU21-04	0	0	9	12	16	25.3	183	47	338	52	213	60
NUCUSU21-05	0	0	7	15	27	34.7	104	63	278	78	197	65
NUCUSU21-06	0	0	5	11.5	13	29.2	73	67	274	75	196	70
NUCUSU21-07	0	0	5	12	17	38	66	50	276	69	178	65
NUCUSU21-08	0	0	6	10.6	43	37	115	46	331	65	235	65

Table: 4- Mean performance of total flower appearance

Entry No	Vine	Flower				Fruit			Yield	Seed	Disease Tolerance	
	Vigour	Flower color	Ovary Pubescence	Ovary color	Sex	Color	Shape	Bitterness		Size	Virus	Other
NUCUSU21-01	Medium	Yellow	Present	Green	PC	Green	MI Cly.	No	Good	Medium	CMV	PM &DM
NUCUSU21-02	Vigorous	Yellow	Present	Green	PC	Light green	ML	No	Good	Medium	No	PM &DM
NUCUSU21-03	Poor	Light yellow	Present	Green	PC	D green	ML	No	Good	Medium	No	PM &DM
NUCUSU21-04	Medium	Light Yellow	Present	Green	PC	Green	ML	No	Good	Medium	No	PM &DM
NUCUSU21-05	Medium	Yellow	Present	Green	PC	Light green	ML	No	Good	Medium	CMV	PM &DM
NUCUSU21-06	Vigorous	Yellow	Present	Green	PC	Green	Long	No	Good	Medium	No	No
NUCUSU21-07	Vigorous	Light Yellow	Present	Green	PC	D green	Long	No	Good	Medium	No	No
NUCUSU21-08	Vigorous	Yellow	Present	D Green	PC	Green	V Long	No	Good	Medium	CMV	No

Table: 5- Quantitative characteristics of genotype

Entry No	Vine			Leaf			Flower			Fruit		Yield	
	Length (cm)	No. Primary Branches	Inter node distance	Length (CM)	Width (CM)	Petiole length (CM)	1st flower	50 % flower	Ovary length (CM)	Fruit length (CM)	Fruit Dia. (CM)	Avg. Fruit wt.	No. of fruits/plant
NUCUSU21-01	180	6	8	20	24	15	36	37	3	20	5	200	103
NUCUSU21-02	230	9	7.5	15	21	10	34	45	4.2	23	5	250	110
NUCUSU21-03	140	3	6	17	24	13	48	58	3.1	16	4	210	98
NUCUSU21-04	170	7	7	17	23	14	49	58	3.4	19	4	200	125
NUCUSU21-05	150	3	8.5	18	21	18	29	34	4.5	18	4	200	93
NUCUSU21-06	210	4	7	17	22	15	36	41	5	26	4	280	80
NUCUSU21-07	205	6	8	21	23	16.5	32	53	3.8	16	4	120	110
NUCUSU21-08	200	4	10	20	24	21	33	35	4	20	4	140	112

Table: 6- Qualitative observation of genotype

Data pertaining to the effect of varying concentrations of silver nitrate on gynoecious cultivars have been presented in Table: -4. Higher concentrations of silver nitrate adversely affected the overall growth of plants and mean comparisons showed that silver nitrate had a significant effect on the number of staminate flower induction whereas no male flower has been noticed in the control treatment (Distilled water). The present findings are corroborated by the study of Hirayama and Alonso (2000), Chaudhary et al., (2001), Law et al., (2002), Stankovic and Prodanovic (2002), Hallidri (2004), Karakaya and Padem (2011), Golabadi et al., (2015), Meena (2015) and Prajapati et al., (2015). They also reported that silver nitrate at higher concentrations was phytotoxic and produced a burning effect in leaves and exhibited highly retarded growth of plants.

3.2. The Effects of AgNO₃ on the number of female flowers

The female flowers were observed both in the control and AgNO₃ applied plants. The differences between the numbers of female flowers having applied different AgNO₃ doses to the variables were determined to be statistically significant. It was determined that the number of female flowers decreased depending on the increased AgNO₃ doses applied to the varieties.

3.3. The effect of AgNO₃ on Seed development

It was determined that the number of seed per fruit depend on good quality pollen and so, we can say that the increasing dose of AgNO₃ not only increasing no of flower but also producing good quality pollen, Good pollen quality produce good quality and sound seed g on the increased AgNO₃ doses applied treatment, Controlled (no seed), 1000 ppm NUCUSU21-02 & NUCUSU21-03 (75) , NUCUSU21-06 (70), NUCUSU21-05, NUCUSU21-07 & NUCUSU21-08 (65), NUCUSU21-04 (60), NUCUSU21-01 (50) 800 ppm NUCUSU21-02 (80), NUCUSU21-05 (78), NUCUSU21-06 (75), NUCUSU21-03 (71), NUCUSU21-07 (69), NUCUSU21-08 (65), NUCUSU21-01 (63) & NUCUSU21-04 (52) 600 ppm NUCUSU21-06 (67), NUCUSU21-05 (63), NUCUSU21-03 (60), NUCUSU21-01 (55), NUCUSU21-07 (50), NUCUSU21-02 (48), NUCUSU21-04 (47), & NUCUSU21-08 (46) 400ppm NUCUSU21-07 (38) NUCUSU21-08 (37) NUCUSU21-02 (36) NUCUSU21-05 (34.7), NUCUSU21-01 (30), NUCUSU21-03 (29.6), NUCUSU21-06 (29.2), & NUCUSU21-04 (25.3) 200ppm NUCUSU21-02 (22.2), NUCUSU21-03 (18), NUCUSU21-05 (15), NUCUSU21-07 & NUCUSU21-04 (12), NUCUSU21-06 (11.5) NUCUSU21-06 (11.5) NUCUSU21-08 (10.6)

NUCUSU21-01 (10) and the differences were determined to be statistically significant.

4. Conclusion

Finally, the 800 ppm concentration of silver nitrate (AgNO₃) chemical were found to be superior to other concentration of AgNO₃ e.g. 1000 ppm found phytotoxic where 600 ppm 400 ppm & 200 ppm lower than 800 ppm, with respect to male flower production; hence, 800 ppm could be recommended for enhancing male flower production in cucumber. The findings showed that the highest number of male flowers was induced by applying AgNO₃ (800 ppm). Based on the results obtained for the different doses treatments, the 3 spray is recommended to start at the 3- 6 and 15-leaf stages.

Authors' contribution

Conceptualization of research: - Shlok Bhalinge & Rajan Prasad Mishra

Designing of experiments: - Rajan Prasad Mishra

Execution of field/lab experiments and data collection: - Gyan singh & Rohidas A W.

Analysis of data and interpretation: - Rajan Prasad Mishra, Sandeep Kumar & Ashok Kumar

Preparation of the manuscript: - Rajan Prasad Mishra

Acknowledgement

Special thanks to Mr. Sachin Bhalinge (Managing Director NUA IPL) & Mr. Ganesh Ladkad And entire R&D team.

REFERENCES

1. Cantliffe DJ (1981). Alteration of sex expression incucumber due to changes in temperature light intensity and photoperiod. J Amer Soc Horti Sci 106:133-136.
2. Deakin, J.R., G.W. Bohn, and T.W. Whitaker. 1971. Interspecific hybridization in Cucumis. Econ. Bot. 25:195-211.
3. Dhiman and Prakash, C. 2005. Correlation and path coefficient analysis in cucumber. Haryana J. Horti. Sci., 34(1- 2): 111-112.
4. Dhatt, A.S. and G. Kaur: Parthenocarpy: A potential trait to exploit in vegetable crops: Areview. Agril Rev., 37, 300-308 (2016).
5. Golabadi, M., Golkar, P. and Egthedari, A.R. 2015. Use of chemical and hormonal agents for changing sex expression of cucumber for breeding programs. Biharean Biologist (online first): art.151417.
6. Hallidri, M. (2004): Effect of silver nitrate on induction of staminate flowers in gynoecious

7. Cucumber line (*Cucumis sativus* L.). ISHS Acta Horticulture, 637: XXVI International Congress
8. Advances in Vegetable Breeding, 31 May 2004, Toronto, Canada.
9. Hirayama, T., Alonso, J. M. (2000): Metal ions are involved in ethylene perception and signal transduction. *Plant Cell Physiology* 41(5): 548-555.
10. Kumar, S., Thappa, M. and Rafiq, R. 2011. Influence of plant growth regulators on morphological, floral and yield traits of cucumber (*Cucumis sativus* L.). *Kasetsart Journal of Natural Science*. 45:177-188.
11. Law, T.F., Lebel-hardenack S Grant, S.R. (2002): Silver enhances stamen development in female white Campion (*Silene latifolia* [Caryophyllaceae]). *American Journal of Botany* 89: 1014-1020.
12. Mehdi, M., Ahmed, N., Jabeen, N., Khan, S.H. and Afroza, B. 2012. Effect of ethrel on hybrid seed production of cucumber (*Cucumis sativus* L.) under open and protected conditions. *The Asian Journal of Horticulture*. 7:558-560.
13. Meena, O.P. 2015. Role of plant growth regulators in vegetable production. *International Journal of Agricultural Science and Research*. 5:71-84.
14. Pike, L.M. and Peterson C.E. 1969. Inheritance of parthenocarpy in the cucumber (*Cucumis sativus* L.). *Euphytica*, 18: 101-105.
15. Stankovic, L., Prodanovic, S. (2002): Silver nitrate effects on sex expression in cucumber. *ISHS Acta Horticulture*, 579: II Balkan Symposium on Vegetables and Potatoes. Thessaloniki, Greece.

BIOGRAPHIES



Rajan P Mishra a vegetable breeder professionally. With one decade experience in Vegetable Breeding, specialization in Cucurbits and Tomato. Currently working at Namdeo Umaji Agritech India Pvt. Ltd. Pune, as Senior Breeder Cucurbits.
Mob. - +91 9454253961,
Email- rajanmishrask@gmail.com