

INFLUENCE OF KINETIN ON GROWTH, FLOWERING AND ALKALOIDAL CONTENTS OF *HYOSCYAMUS MUTICUS* L.

S. IFTIKHAR H. SHAH and G.A. MIANA

Department of Pharmacy, Gomal University, Dera Ismail Khan

ABSTRACT

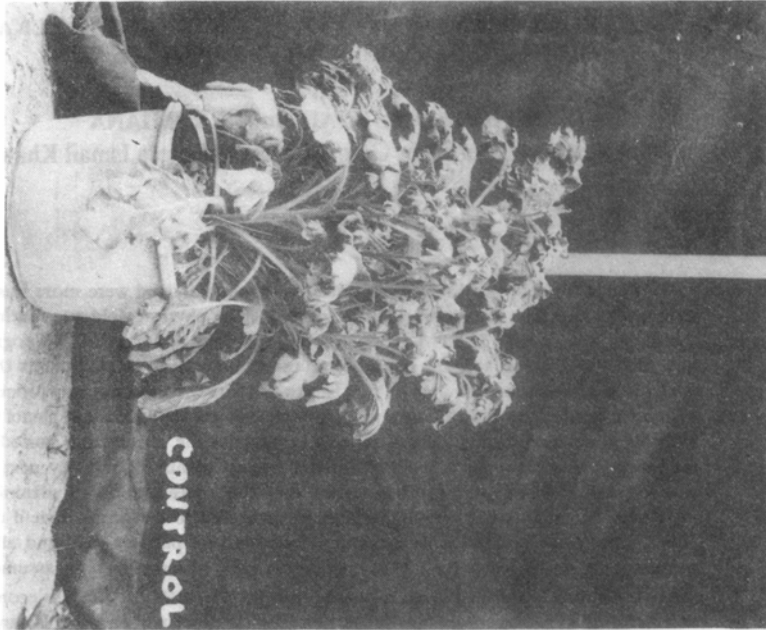
Plants treated with Kinetin responded very markedly and were more bushy and darker in colour than the controls (Photograph-1). The physical yield was maximum in the plants treated with 50 PPM (Photograph-2) followed by 100 PPM (Photograph-3) and 200 PPM (Photograph-4). Early flowering was induced in treated plants than the controls and it was seen lint in 50 PPM. Growth and flowering was not very promising in higher concentration of the Kinetin. Height measurement indicated that plants treated with the Kinetin were larger than controls throughout the observation period. This indication was prominent in 50 PPM Kinetin treated plants followed by next higher concentrations. Chemical assay has shown that the concentration of alkaloids has increased though it is not very appreciably as compared to the physical yield of each plant. Lower concentrations have better effects both on physical yield and alkaloids percentage as compared to the higher ones. From these observations it is assumed that the use of Kinetin as plant growth regulator on *Hyoscyamus muticus* is economical and its further investigations may prove more useful especially on other solanaceous plants.

Introduction

Kinetin, 6-(Furfuryl amino)-purine is known as a plant growth regulator and has profound effect on cell division, cell enlargement, shoot formation, root development and seed germination. It has also shown an increase in alkaloid synthesis and nitrogen metabolism (Ambrose and Scicchetti 1962, Miller *et al.*, 1955).

A review of literature has indicated that very little attention has been paid to investigate the effect of this substance on medicinal and economic plants. Keeping this in view, the present work carried out to study the effect of Kinetin on the growth, development and alkaloidal pattern of *Hyoscyamus muticus* at different physiological stages.

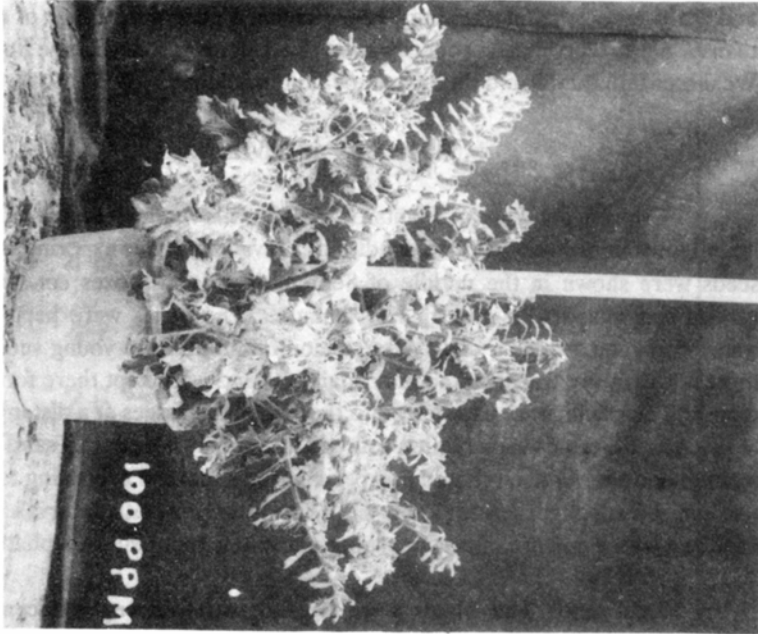
Hyoscyamus muticus is indigenous in Pakistan and is found scattered in Kurram Valley, North Waziristan, Swat, Chitral and Tribal territories. It is common in Kaghan Valley, Azad Kashmir and Gilgit Agency. The main macroscopic characters of the drug are: longitudinally strained stem, pale green to yellowish brown leaves, fruit a pyxidium and minute seeds with reticulate testa (The Wealth of India 1959).



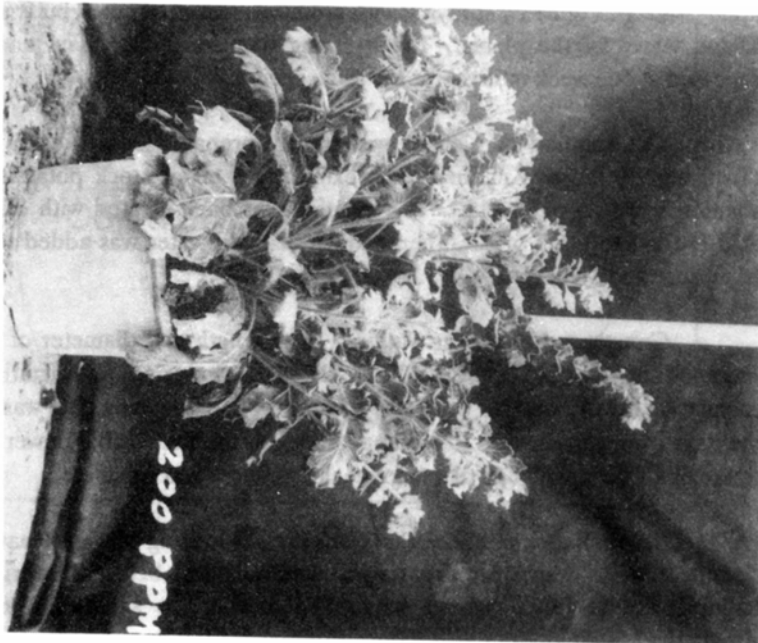
Photograph-1
Hyoscyamus muticus
Expt. = 1
Control
Hgt: 24"



Photograph-2
Hyoscyamus muticus
Expt. = 1
50 ppm
Hgt: 28"



Photograph-3
Hyoscyamus muticus
Expt. = 1
100 ppm
Hgt: 26"



Photograph-4
Hyoscyamus muticus
Expt. = 1
200 ppm
Hgt: 25"

The herb is generally in matted masses of leaves intermixed with stems and flowering tops and also a few fruits; odour slightly foetid and narcotic; taste bitter and acrid especially on chewing. It contains a higher percentage of total alkaloids than *H. niger*. The highest concentration of alkaloids is found in the floral parts (The Wealth of India, 1959 and Pharmacopoea Internationalis, WHO 1951).

Materials and Methods

Growing the Plants:- Plants of *Hyoscyamus muticus* employed in this study were grown in ordinary climate conditions prevailing at our experimental farm. The seeds were sown in the middle of September in seed boxes containing ordinary garden soil mixed with enough leaf manure. The boxes were kept in shade and watered regularly once a day. After 1 month, the uniform young seedlings (two to three leaves) were transplanted into small pots and were kept there for an additional month. The plants were transferred to the soil on the ridges at a distance of about 40 to 45 cms. Before transplantation soil was thoroughly mixed with sand and cowdung manure and the whole area under experiment was marked according to the attached randomized block diagram (Table-1). Then after 1 month intervals, the soil was fertilized with a mixture of calcium super phosphate, potassium sulphate and urea.

Treatment- The plants were treated with different concentrations of a freshly prepared aqueous solution of Kinetin (50, 100 and 200 PPM). The application was done by spraying with atomizer on the terminal buds and the young folded leaves of the plants. The spraying was done for 6 days and then it was repeated at one week interval for the same period.

The solution of Kinetin was freshly prepared before each treatment by dissolving 2.5 mg of Kinetin in approximately 3 ml of stock potassium hydroxide solution having a pH of 11.2. This solution was back titrated with dilute sulphuric acid ($10^{-3}N$) to a pH of 6.5. Then enough distilled water was added to make 10 (Ambrose and Sciuchetti, 1962).

Growth and Flowering Studies:- The length and diameter of the stem recorded at 10 days interval (Table-2). The flowering and fruiting dates for calculating the dry weights were also recorded. The flowering date was calculated the days from germination to the appearance of the first visible flower bud for treatment. The mean of 10 plants for each treatment was calculated.

Determination of Alkaloidal Contents:- Flowering tops and leaves from treatment were drawn for dry weight determination before flowering, at fl budding and at full flowering stages (Table-3). The samples were dried in circulating hot air oven at 70°, reduced to powder (40 mesh) and stored in air containers for the estimation of hyoscyamine. The T.C.P. method with s modifications (The Indian Pharm. Codex 1953) was adopted for the chemical assay.

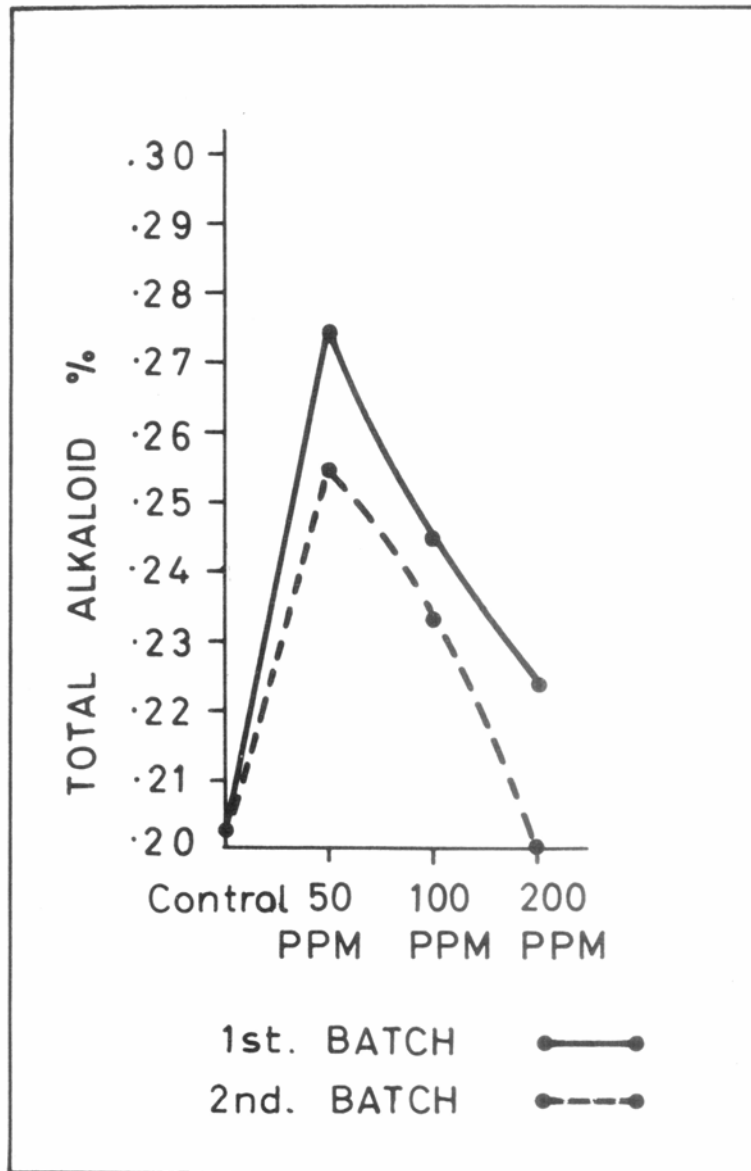


Figure 1.

Influence of kinetin at various concentration on the alkaloidal contents.

Table 1Influence of kinetin on vegetative growth of *Hyoscyamus muticus*.

Plant characteristics	Concentration of Kinetin PPM	Plant Age (days)									
		135	145	155	165	175	185	195	205	215	Mean
Stem length (cm/plant)	00	5.3 ^{a*}	6.5	10.3	15.0	26.6	35.2	42.4	51.7	55.0	27.55
	50	9.2	12.6	18.5	27.1	38.7	49.4	60.1	71.8	80.6	40.88
	100	8.1	10.4	17.2	24.4	36.3	46.1	51.3	62.2	69.4	36.15
	200	6.4	9.0	14.1	19.6	25.7	33.4	41.8	52.3	61.9	29.35
	MEAN	7.2	9.6	15.25	21.52	31.82	41.02	48.90	59.50	66.72	-
Stem Diameter (cm/plant)	00	1.3 ^{a*}	1.3	1.4	1.5	1.5	1.7	1.8	1.8	1.9	1.57
	50	2.3	2.3	2.4	2.5	2.5	2.6	2.7	2.8	2.8	2.54
	100	1.8	1.8	1.9	1.9	2.0	2.1	2.3	2.4	2.4	2.06
	200	1.5	1.6	1.6	1.7	1.8	1.8	1.9	2.0	2.0	1.76
	MEAN	1.72	1.75	1.82	1.90	1.95	2.05	2.17	2.25	2.27	-
Randomized Block Design											
Main Irrigation Channel	200 PPM	100 PPM		00 PPM		50 PPM					
	50 PPM	00 PPM		100 PPM		200 PPM					
	00 PPM	50 PPM		200 PPM		100 PPM					

Randomized block design:-

Cross plot size = 1 Kanal = 5445 Sq. ft. = 99x55 ft.

Net plot size = 25x 18 Sq. ft.

*a = Average of 10 plants per treatment.

Table 2*Stem length and stem diameter analysis of variation table*

Sources of Variation	Degree of Freedom		Sum of Squares		Mean sum of Squares		F.Ratio	Remarks	
	Stem Length	Stem Dia.	Stem Length	Stem Dia.	Stem Length	Stem Dia.	Stem Length	Stem Dia.	
Treatment	3	3	1179.28	1.80	393.09	0.60	274.89	60.00	Highly
Replicate	2	2	2.17	0.05	1.09	0.02	00.76	-	Significant.
Error	6	6	8.56	0.03	1.43	0.01	-	-	

Standard Error:

Stem Length = 0.5636 cm/plot.
Stem Diameter = 0.0470 cm/plot.

$H_0 = T_0 = T_1 = T_2 = T_3$
 $H_1 = T_0 = T_1 = T_2 = T_3$

According to ANOVA Table the result of treatment are highly significant.

Least significant difference test (LSD):

Stem Length = 1.38
 $T_0 = 53.68, T_3 = 61.97, T_2 = 69.50, T_1 = 80.62$

Stem diameter = 0.115
 $T_0 = 1.85, T_3 = 1.97, T_2 = 2.42, T_1 = 2.85$

No significant difference between untreated and treated plants with Kinetin at 200 PPM but Kinetin at 50 PPM is highly effective.

* $T_0 = \text{Control}, T_1 = 50 \text{ PPM}, T_2 = 100 \text{ PPM}, T_3 = 200 \text{ PPM}.$

Table 3Influence of kinetin on dry wt. and alkaloidal contents of *Hyoscyamus muticus*.

Stage of Growth	KINETIN				CONCENTRATION		PPM	
	00		50		100		200	
	D.W.	A.C.	D.W.	A.C.	D.W.	A.C.	D.W.	A.C.
Before Flowering	3.70*	.201*	5.98	.269	4.17	.238	3.62	.207
At Flower Budding	12.27	.213	20.03	.276	16.64	.242	13.94	.225
At Full Flowering	33.00	.210	63.82	.268	45.23	.240	38.01	.204
At Fruiting	65.00	-	93.61	-	78.10	-	69.00	-
MEAN%		.208		.271		.240		.212

* Average based on three replicates per treatment.
D.W. = Dry weight
A.C. = Alkaloidal Content

Results and Discussion

Vegetative Growth:- It has been observed that some metabolic effects have taken place in the plants treated with Kinetin and several differences were noted at the various stages throughout the growth period (Seth *et al*, 1966 and Raddin and Loomis, 1974). Stem length, diameter, number of leaves and branches were significantly increased by treatment with Kinetin especially at 50 PPM (Table-2, & photograph). This stimulatory effect was pronounced at the *early* stages of growth and continued at a decreasing rate until the end of growth. There was significant increase in the dry weight of the flowering tops and shoots especially at full flowering stage (Table-3) than controls. This result clearly indicates that the physiological stage may play an important role in obtaining a good response from Kinetin treatment.

The increase in the dry weight of the shoots is the result of the increased number of the branches and leaves and this response may be attributed to the increase in carbon rotation of Kinetin and to the redistribution of materials from the roots to the shoots.

Flowering and Fruiting response:- It has been observed that the treated plants significantly enhanced both the processes flowering and fruiting as compared with the controls. The 50 PPM Kinetin treated plants yielded flowers 10 days and fruits 7 days earlier than the controls. The flowering rate was increased for all treatments during the first period of the flowering season. These results show that Kinetin plays an indirect and important role in the stimulation and promotion of the early flowering process which results in increase in the number of fruits especially treated with 50 PPM.

Alkaloidal Analysis:- From the chemical assay it is noticed (Figure 1) that the alkaloidal contents of shoots of treated plants was increased at different stages of growth (Ahmad and Malik, 1965).

A significant increase in alkaloidal contents of shoots was recorded for the plants treated with 50 PPM Kinetin, especially at flowering stages. This increase in alkaloidal contents due to Kinetin treatment was the result of an indirect influence of the stimulation of the vegetative growth, flowering process and metabolic activity of the plants.

References

- Ahmad, A.J., and Malik, N.A. (1965). Effect of G.A. on growth and Alkaloids in *Hyoscyamus muticus*. *Pakistan J. Sci. Ind. Res.* **8**, 65.
- Ambrose, D.G., and Sciuchetti, Leo A. (1962). Influence of Kinetin and Gibberellic acid on the growth and Alkaloid Patterns in *Datura meteloides*. *Jr. Phar. Sci.* **51**, 934.
- Miller, C.O., Shoog, F., Von Soltza, M.H., and Strong, F.M. (1955). Kinetin a cell division factor from Deoxyribonucleic acid. *J. Am. Chem. Soc.* **77**, 1392.
- Pharmacopoea Internationalis WHO. (1951). *Geneva*, Vol-1, 118.

- Pieniazek, J. (1964). Kinetin induced breaking of dormancy in 8-month old apple seedlings of "Antonovka" variety. *Acta Agrobot (Warsaw)* **16**, 157.
- Raddin, AT, and Loomis, R.S. (1974). Polar transport of Kinetin in tissues of Radish. *Plant Physiology* **53**, 348.
- Seth, A .K., Davies, C.R., and Woreing, P.F. (1966). Auxin effect on the mobility of Kinetin in the plant. *Science* **151**, 587.
- Skinner, C.G., Tolbert, F.D. and Shive, W (1958)- Effect of 6- (substituted)-purines and Gibbercllin on the rate of seed germination. *Plant physiology.* **33**, 190.
- Skinner, C.G., and Shive, W. (1957). Effect of some isomeric patine analogues on germination of lettuce seed. *Plant physiology* **32**, 500.
- The Indian Pharm. Codex CSIR (1953). *New Dehli*. Vol. 1, 121.
- The Wealth of India (1959). (raw materials) *CSIR, New Dehli*, Vol.4, H.K. 153.