

## Effect of soaking time of cow urine and rooting media on seed germination of Kagzi lime (*Citrus aurantifolia* Swingle)

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### Abstract

The experiment was conducted to study the effect of soaking time of cow urine and rooting media on seed germination of Kagzi lime. Twenty-one treatment combinations consisting of three main plot treatments of cow urine C<sub>0</sub> (seed soaked in distilled water for 12 hrs.), C<sub>1</sub> (Seed soaked in 10% cow urine for 24 hrs.) and C<sub>2</sub> (Seed soaked in 10% cow urine for 12 hrs.). Seven sub plot treatments of rooting media M<sub>0</sub> (only soil), M<sub>1</sub> [soil + FYM (3:1)], M<sub>2</sub> (Soil + vermicompost (3:1)], M<sub>3</sub> [soil + FYM (1:1)], M<sub>4</sub> [soil + vermicompost (1:1)], M<sub>5</sub> [soil + FYM (1:3)] and M<sub>6</sub> [soil + vermicompost (1:3)] were replicated thrice in split plot design. The observations were recorded replication wise by selecting five random plants for seven parameters, which includes seed germination and seedling growth. The results revealed that minimum days taken to start germination, taken to 50% germination and maximum percentage of germination was observed in (C<sub>1</sub>) seed soaked in 10% cow urine for 24 hrs. Similarly, the treatment C<sub>1</sub> significantly increased the growth characters viz., number of roots/seedling, height of seedling, number of leaves/seedling and leaf area index. As regards the rooting media, M<sub>6</sub> significantly influences all the parameters. It was also superior to rest of the rooting media. Among the interactions of cow urine and rooting media, no any interaction was found significantly superior, but C<sub>1</sub>M<sub>6</sub> treatment combination gave superior results over remaining treatment interactions.

**Key words:** Cow urine, rooting media, kagzi lime, F.Y.M, vermicompost, germination

### Introduction

Kagzi lime (*Citrus aurantifolia* Swingle) is one of the most important tropical and subtropical fruit after than Mandarin and Sweet orange of rutaceae family. In India, Kagzi lime occupies, 2,58,000 hectare area with the annual production of 2.569 million tonnes (Anonymous, 2014). Due to, which there is tremendous scope of this crop to capture the market throughout the year. It is successfully grown in state of Andhra Pradesh, Maharastra, Tamil Nadu and Madhya Pradesh. In M.P., it is mostly grown as commercial crop in east and west Niwar and some part of Malwa and Mahakaushal region.

Lime is usually propagated by seeds and maximum germination can be obtained by sowing of freshly extracted seed up to 2 days only, which are polyembryonic in nature. The seedlings are almost true to the type and free from virus infection. Raising

seedlings of Kagzi lime is often associated with many problems. Under field condition, it is common observation that the seed germination is poor and secondly the seedling growth is slow. The rate of mortality is also very high due to which it is difficult to raise a large population. Plant takes about 1½ to 2 years duration for planting in orchards due to slow growth rate of seedling (Singh *et.al.*, 2004). There are some difficulties in the cultivation of the crop and the present investigation has been undertaken with an intense urged to overcome them.

In recent year, lots of research work has been done on different aspects of growth regulators. The important aspects among them are the most appropriate growth regulators for encouraging germination and further growth. To overcome this crisis some alternative for growth regulator should be brought up which may be as efficient as growth regulators, easy to access and cheap. This has diverted the attention once again towards the chemical, cane sugar, cow urine, which were in use as a growth regulators. Cow urine proving feasible may

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bring a breakthrough in the present context as it is free of cost and easily available. Cow urine contains nitrogen, sulphur, ammonia, copper, iron, urea, uric acid, phosphate, sodium, potassium, manganese, carbolic calcium, salt, vitamins, lactose, enzyme, water, creatinin, aurum hydroxide etc. (Sankaranarayana *et.al.*, 1994). In view of above facts, the experiment was conducted to study the effect of soaking time of cow urine and rooting media on seed germination and seedling growth of Kagzi lime.

### Materials and Methods

The experiment was conducted at Fruit Research Station, Imalia, Department of Horticulture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.). In this experiment the response of cow urine and different rooting media was evaluated for germination and vegetative growth of Kagzi lime seeds. The experiment was laid out in split plot design with three replications. For rooting media, soil, FYM and Vermicompost are obtained. Polybags of 20 cm length and 30 cm diameter with 200 gauge thickness were used. Fully ripened and healthy fruits of uniform size were taken and seeds were extracted carefully. Extracted seeds were washed in running water and dried under shade condition. Cow urine was taken from desi breed at morning time. Ten treatments combination consisting of three main plot treatments of cow urine  $C_0$  (seed soaked in distilled water for 12 hrs.),  $C_1$  (Seed soaked in 10% cow urine for 24 hrs.), and  $C_2$  (Seed soaked in 10% cow urine for 12 hrs.) and seven sub plot treatments of rooting media  $M_0$  (only soil),  $M_1$  [soil + FYM (3:1)],  $M_2$  (Soil + vermicompost (3:1)],  $M_3$  [soil + FYM (1:1)],  $M_4$  [soil + vermicompost (1:1)],  $M_5$  [soil + FYM (1:3)] and  $M_6$  [soil + vermicompost (1:3)] were replicated thrice in split plot design.

Three treated seeds were sowed at 1-2 cm depth in polythene bags which were consisted in different rooting media. After germination only one seedling was retained, other was removed. Intermittent hand weeding was done as and when needed. Roger (0.2%) was sprayed thrice i.e. after emergence of seedling, one month after emergence and two months after emergence. 1% urea was sprayed twice on the seedling once 60 days after sowing. The standard horticultural practices were adopted for raising the seedlings. Parameters regarding seed germination and growth were recorded viz., Days taken to start germination, Days taken to 50% germination, Percentage of germination, Height of seedling (cm.) Number of leaves/seedling, Leaf area index ( $\text{cm}^2$ ) and number of roots per seedling. The data collected during the investigation were analyzed statistical by the method of "Analysis of variance". The significance of various treatments was judged as suggested by Gomez and Gomez (1984) applying "F" Test.

## Results and Discussion

### Days taken to start germination

The data taken pertaining to days to start germination was recorded and statistically analyzed. A perusal of data presented in Table 1 indicates that treatment of cow urine and rooting media significantly decreased the days taken to start germination. Under this experiment,  $C_1$  (seed soaked in 10% cow urine for 24 hrs.) recorded significantly minimum days taken to start germination after sowing (13.33) followed by  $C_2$  (seed soaked in 10% cow urine for 12 hrs.), which recorded 13.76.  $C_0$  (control) taken significantly maximum days to start germination, which is at par with  $C_2$ . As regards the rooting media,  $M_6$  [soil + vermicompost (1: 3)] was recorded the significantly minimum days taken to start germination (12.55) followed by  $M_5$  [soil + FYM (1: 1)] and  $M_4$  [soil vermicompost (1:1)] which recorded 13.11 and 13.44 respectively and the treatment did not defer significantly each other.  $M_0$  (only soil) showed significantly maximum days taken to start germination, which is at par with  $M_1$ . It is emphasized from the table that interaction of cow urine and rooting media are found non significant, however minimum days taken to start germination 11.66 was noted under the combination of  $C_1M_6$  [seed soaked in 10% cow urine for 24 hrs and soil + vermicompost (1:3)]. The results are in conformity to the results as reported by Rajwar *et al.*, (2007) in case of wild Ber (*Zizyphus rotundifolia*).

### Days taken to 50% germination

The tabulated result clearly shows that treatments of cow urine and rooting media significantly influence the days taken to 50% germination (Table 2). It indicates that the cow urine  $C_1$  (seed soaked in 10% cow urine for 24 hrs.) recorded significantly minimum days taken to 50% germination (18.42) after sowing followed by Cow urine  $C_2$  (seed soaked in 10% cow urine for 12 hrs.), which recorded 19.85 days taken to 50% germination, while  $C_0$  (control) recorded significantly maximum (20.76) days taken to 50% germination. Among the rooting media,  $M_6$  [soil + vermicompost (1:3)] taken significantly minimum days to 50% germination (16.55), followed by  $M_5$  [soil + FYM (1:3)]. The probable reason may be that the medium  $M_6$  creates sufficient porous space, better drainage of water and sufficient aeration for better rooting of seed or may be due to increased physiological activities of seed, essential for cell division, cell enlargement or both. These results are in conformity with the result of Deol and Uppul, (1990).  $M_0$  (only soil) recorded the significantly maximum days taken to 50% germination (23.00), which is at par with  $M_1$  [soil + FYM (3:1)]. As regard interaction, the interaction of cow urine and media are found non

significant, however, minimum days taken to 50% germination (15.00) was recorded under the combination of C<sub>1</sub>M<sub>6</sub> [seed soaked in 10% cow urine for 24 hrs and soil + vermicompost (1:3)]. Similar findings have already been reported by Bertocci *et al.*, (1997) in germination response of Papaya.

### Percentage of germination

Pertaining to percentage of germination was recorded at 30 days after sowing and data was statisticalil analyzed. The results elucidated in Table 3 indicates that the treatment of cow urine and rooting media significantly increased the percentage of germination. It is clear from the statistical data that maximum (67.29) percentage of germination was observed under C<sub>1</sub> (seed soaked in 10% cow urine for 24 hrs.) at 30 days after sowing followed by C<sub>2</sub> (seed soaked in 10% cow urine for 12 hrs.), which recorded 63.4 but not significant better than control. Increase percentage of germination due to cow urine may be due

to fact that perhaps cow urine activated the embryos and estimated the release of enzyme in endosperm, which in turn liberate the food substances required by quick growing embryo and seedling. These findings are in agreement with results of Pandey and Singh (2000) in germination of guava seeds. In case of rooting media, M<sub>6</sub> [soil + vermicompost (1:3)] recorded significantly maximum (80.70) percentage of germination followed by M<sub>5</sub> [soil + FYM (1:3)] and M<sub>4</sub> [soil + vermicompost (1:1)], which recorded 74.07 and 72.22, respectively. M<sub>0</sub> (control) showed significantly minimum percentage of germination 45.92 followed by M<sub>1</sub> [soil + FYM (3:1)] 52.21. However, maximum germination percentage (84.44) was recorded under the combination of C<sub>1</sub>M<sub>6</sub> [seed soaked in 10% cow urine for 24 hrs and soil + vermicompost (1:3)]. More or less similar results have been reported by Singh, *et al.* (2000).

### Height of seedlings

An effect of application of cow urine and

Table 1. Effect of soaking time of cow urine and rooting media on days taken to start germination of Kagzi lime.

Treatments	Media							Mean
Cow urine	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>4</sub>	M <sub>5</sub>	M <sub>6</sub>	
C <sub>0</sub>	16.3	15.66	15.0	14.6	14.0	13.6	13.33	14.66
	3		0	6	0	6		
C <sub>1</sub>	15.0	13.66	13.6	13.3	13.0	12.6	11.66	13.33
	0		6	3	0	6		
C <sub>2</sub>	15.3	14.66	13.6	13.6	13.0	13.0	12.66	13.76
	3		6	6	0	0		
Mean	15.5	14.77	14.1	13.8	13.4	13.1	12.55	
	5		1	8	4	1		
Treatments		S.Em±			C.D at 5% level			
C (cow urine)		0.24			0.98			
M (media)		0.36			1.03			
CxM (interaction)		0.67			NS			

Table 2. Effect of soaking time of cow urine and rooting media on days taken to 50% germination of Kagzi lime.

Treatments	Media							Mean
Cow urine	M <sub>0</sub>	M	M	M <sub>3</sub>	M	M	M <sub>6</sub>	
		1	2		4	5		
C <sub>0</sub>	24.00	22.66	21.66	20.66	19.33	19.00	18.00	20.76
C <sub>1</sub>	22.00	20.66	19.33	18.00	17.33	16.66	15.00	18.42
C <sub>2</sub>	23.00	21.00	20.66	20.33	18.66	18.66	16.66	19.85
Mean	23.00	21.44	20.55	19.66	18.44	18.11	16.55	
Treatments		S.Em±			C.D at 5% level			
C (cow urine)		0.150			0.57			
M (media)		0.60			1.70			
CxM (interaction)		1.031			N.S			

Table 3. Effect of soaking time of cow urine and rooting media on percentage of germination of Kagzi lime.

Treatments	Media							Mean
Cow urine	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>4</sub>	M <sub>5</sub>	M <sub>6</sub>	
C <sub>0</sub>	42.21	47.77	54.44	59.99	70.00	71.10	77.66	60.45
C <sub>1</sub>	49.99	56.66	62.21	66.66	74.44	76.66	84.44	67.29
C <sub>2</sub>	45.55	52.21	56.66	63.33	72.22	74.44	79.99	63.40
Mean	45.92	52.21	57.77	63.33	72.22	74.07	80.70	
Treatments		S.Em $\pm$			C.D at 5% level			
C (cow urine)		0.94			3.70			
M (media)		1.88			5.40			
CxM (interaction)		3.26			NS			

rooting media significantly influenced the vegetative growth of plant. The data on the height of seedlings were recorded at 60, 75, 90, 105, 120 and 135 days after sowing (Table 4). Among the different concentrations of cow urine, C<sub>1</sub> (seed soaked in 10% cow urine for 24 hrs.) recorded the significantly higher plant height (6.45, 7.45, 8.60, 9.55, 10.51 and 11.76 cm at 60, 75, 90, 105, 120 and 135 days after sowing respectively), followed by C<sub>2</sub> (seed soaked in 10% cow urine for 12 hrs.) which recorded 5.94, 6.98, 8.00, 8.93, 9.80 and 10.82 cm at various stages respectively.

This may be due to Cow urine seems to affect physiochemical characteristics of seed and thus give positive response to germination, emergence, vigour and plant height of seedlings. Similar results were also recorded by Parameswari *et al.* (2001) in tamarind seed. The table 4 further indicates that M<sub>6</sub> [soil +

vermicompost (1:3)] recorded significantly higher plant height (8.05, 9.17, 10.41, 11.53, 12.56 and 13.93 cm at various stages, respectively), followed by M<sub>4</sub> [soil + vermicompost (1:1)] might be due to vermicompost which had supplied available plant nutrients and brought about a favorable soil environment, which ultimately increased the nutrient and water holding capacity of soil. It is much closed related with Kumar and Satyawati (2007) in *Jatropha curcus*. Whereas, M<sub>0</sub> (control) reported significantly minimum height of seedling (4.08, 4.69, 5.39, 5.88, 6.30, and 7.05) at various stages, respectively followed by M<sub>1</sub> [soil + FYM 3:1)].

#### Number of leaves/seedling

The data pertaining to number of leaves/seedling was recorded after 60 to 135 days after sowing

Table 4. Effect of soaking time of cow urine and rooting media on height of seedling at various stages of Kagzi lime.

Treatments	Days after sowing					
Cow urine	60	75	90	105	120	135
C <sub>0</sub>	5.43	6.38	7.33	8.26	9.11	9.85
C <sub>1</sub>	6.45	7.45	8.60	9.55	10.51	11.76
C <sub>2</sub>	5.95	6.98	8.00	8.93	9.80	10.82
SEM $\pm$	0.04	0.04	0.05	0.05	0.07	0.13
C.D at 5% level	0.17	0.18	0.21	0.22	0.25	0.52
Media						
M <sub>0</sub>	4.08	4.69	5.39	5.88	6.30	7.05
M <sub>1</sub>	5.06	5.95	6.96	7.85	8.71	9.61
M <sub>2</sub>	5.43	6.48	7.50	8.45	9.29	10.24
M <sub>3</sub>	5.64	6.88	7.89	8.91	9.85	10.62
M <sub>4</sub>	6.84	7.85	9.01	10.03	11.11	12.24
M <sub>5</sub>	6.50	7.54	8.70	9.76	10.83	12.00
M <sub>6</sub>	8.05	9.17	10.41	11.53	12.56	13.93
SEM $\pm$	0.14	0.13	0.14	0.14	0.15	0.23
C.D at 5% level	0.39	0.39	0.41	0.40	0.43	0.65

at a regular interval of 15 days under different treatments of cow urine and rooting media. Among the cow urine,  $C_1$  (seed soaked in cow urine for 24 hrs.) and among rooting media,  $M_6$  [soil + vermicompost (1:3)] produced the maximum number of leaves/seedling at all the successive stages of seedling growth. It is apparent from Table 5 that cow urine  $C_1$  produced significantly more number of leaves seedling 8.30, 11.39, 13.94, 15.84, 18.51 and 21.51 at 60, 75, 90, 105, 120 and 135 days after sowing respectively, followed by  $C_2$ . While  $C_0$  (control) produced significantly minimum number of leaves *i.e.*, 6.83, 9.31, 11.30, 13.34, 15.25 and 17.60 at various stage, respectively sowing. Shaban (2010) who reported that the probable reason of enhances more number of leaves per plant in cow urine treated seed that it might have resulted in more production of photosynthets and their translocation through phloem to the whole plant might be responsible for improving the vegetable growth which finally helps in increase number of leaves per seedling. It further reveals the rooting media,  $M_6$  [soil + vermicompost (1:3)] produced the significantly maximum number of leaves/ Seedling 9.27, 13.50, 17.27, 20.83, 24.43 and 28.38 at various stages, respectively, followed by rest of the rooting media. These results are in consonance with the findings as reported by Verma and Prasad (2005). Among the interactions, the interaction between cow urine and rooting media not produced significantly higher number of leaves/seedling.

### Leaf area index

As far as the leaf area index (LAI) is concerned, maximum ( $2.55 \text{ cm}^2$ ) LAI was found under  $C_1$  (seed soaked in 10% cow urine for 24 hrs.) at 135 days after sowing. The value of LAI increased due to maximum length and width of leaves produced by synergistic effect of cow urine and their movement increased the synthesis of biomass and accumulation of photosynthates that is responsible for higher leaf area index. The improvement of leaf area index in cow urine treated seeds has been reported by Shirol *et al.* (2005) in Khirnee seeds. Among the rooting media,  $M_6$  [soil + vermicompost (1:3)] encouraged significantly larger leaf area index 1.018, 1.513, 1.990, 2.452, 2.828 and  $3.227 \text{ cm}^2$  at 60, 75, 90, 105, 120 and 135 days after sowing, respectively (Table 6). It was superior to rest of the rooting media. Whereas,  $M_0$  (only soil) showed significantly minimum leaf area index at various stages. It is possible due to rapid multiplication of new cells in higher ratio of vermicompost rooting media which helped increasing in term of length and width of leaves as reported by Norman *et al.*, (2003). Vermicopost contains macro and micro nutrients, enzymes, pro vitamins and growth hormones which are very effective in producing the superior length and width of leaves. Similar results were reported by Vijayanathan and Kumar (2006).

### Number of roots/seedling

The number of roots/seedling was measured at 60 and 135 days after seed sowing. The statistical

Table 5. Effect of soaking time of cow urine and rooting media on number of leaves/seedling at various stages of Kagzi lime.

Treatment	Days after sowing					
Cow urine	60	75	90	105	120	135
$C_0$	6.83	9.31	11.30	13.24	15.25	17.60
$C_1$	8.30	11.39	13.94	15.84	18.51	21.51
$C_2$	7.54	10.39	12.61	14.80	16.90	19.67
SEM $\pm$	0.093	0.115	0.20	0.20	0.22	0.19
C.D at 5% level	0.365	0.452	0.79	0.77	0.88	0.75
Media						
$M_0$	5.63	6.90	7.63	8.44	9.03	10.27
$M_1$	6.07	7.53	9.03	9.58	10.50	12.41
$M_2$	7.26	9.21	10.51	12.33	13.80	16.53
$M_3$	7.80	11.05	13.38	15.82	18.34	21.16
$M_4$	8.90	12.74	15.86	17.79	21.57	24.82
$M_5$	7.96	11.60	14.64	17.62	20.54	23.62
$M_6$	9.27	13.50	17.27	20.83	24.43	28.38
SEM $\pm$	0.273	0.271	0.50	0.64	0.76	0.81
C.D at 5% level	0.783	1.066	1.42	1.83	1.93	2.32

Table 6. Effect of soaking time of cow urine and rooting media on leaf area index (cm<sup>2</sup>) at various stages of Kagzi lime.

Treatments	Days after sowing					
Cow urine	60	75	90	105	120	135
C <sub>0</sub>	0.773	1.081	1.362	1.623	1.866	2.161
C <sub>1</sub>	0.922	1.288	1.600	1.914	2.208	2.551
C <sub>2</sub>	0.844	1.185	1.500	1.773	1.989	2.355
SEM ±	0.023	0.013	0.023	0.019	0.043	0.021
C.D at 5% level	0.093	0.051	0.092	0.076	0.172	0.083
Media						
M <sub>0</sub>	0.516	0.685	0.831	0.930	1.063	1.190
M <sub>1</sub>	0.716	0.960	1.222	1.357	1.531	1.725
M <sub>2</sub>	0.837	1.126	1.355	1.567	1.769	2.008
M <sub>3</sub>	0.887	1.243	1.495	1.887	2.182	2.512
M <sub>4</sub>	0.992	1.419	1.873	2.220	2.527	3.010
M <sub>5</sub>	0.958	1.348	1.678	1.990	2.249	2.810
M <sub>6</sub>	1.018	1.513	1.990	2.452	2.828	3.227
SEM ±	0.045	0.042	0.057	0.066	0.087	0.089
C.D at 5% level	0.129	0.121	0.165	0.192	0.250	0.255

analysis of data (Table 7) revealed that the C<sub>1</sub> (seed soaked in 10% cow urine for 24 hrs.) induced the significantly maximum 9.77 and 29.02 number of roots/plant at 60 and 135 days after sowing, respectively. The minimum number of roots were noted under untreated treatment (C<sub>0</sub>) at both stages, which is at par with C<sub>2</sub>. This may be due to disinfectant action of cow urine, which might have avoided the basal portion of seed from decreasing and must have provided congenial environmental conditions for early initial of rooting. These findings are similar to the findings of Kumar *et al.*, (2012). In case of rooting media combination, the media M<sub>6</sub> [soil + vermicompost (1:3)]

showed significantly the maximum 12.48 and 37.99 number of roots at both stages. Whereas, interaction of cow urine and rooting media are not significantly increase number of roots /plant at both stages of growth. The results of the present study are in consonance with the findings of Bisen *et al.*, (2010) who reported that the media M<sub>6</sub> creates sufficient porous space letting the excess water drain away, permitting adequate aeration for the better root length and number of roots/seedling. More or less similar results were also reported by Jain and Parmar (1993) and Arancon *et al.*, (2004) in strawberry.

Table 7. Effect of soaking time of cow urine and rooting media on number of roots/ seedling of Kagzi lime.

Treatments	Days after sowing	
Cow urine	60	135
C <sub>0</sub>	7.96	23.95
C <sub>1</sub>	9.7	29.02
C <sub>2</sub>	8.78	26.42
SEM ±	0.23	0.25
C.D at 5% level	0.89	0.98
Media		
M <sub>0</sub>	4.15	14.68
M <sub>1</sub>	6.16	19.27
M <sub>2</sub>	7.41	23.42
M <sub>3</sub>	9.75	26.87
M <sub>4</sub>	11.28	32.46
M <sub>5</sub>	10.52	30.54
M <sub>6</sub>	12.48	37.99
SEM ±	0.40	0.95
C.D at 5% level	1.15	2.73



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