

## Formulation of Vermiwash and Humic Acid and its Application on *Allium cepa*

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The continuous use of chemical fertilizers is leading to the contamination of soil and adversely affects the flora and fauna. Look out for natural forms of fertilizers is unceasing. The aim of the present investigation was to determine the influence of vermiwash and humic acid and their combinations various concentrations on the growth of *Allium cepa*. Vermiwash and humic acid and combinations of vermiwash and humic acid are used as plant growth promoters in varying concentrations. In this study, the concentration of 3:1.5 % (vermiwash: humic acid) was found to be the most effective formulation which was manifested in increase in growth, soft shoots, number of branches, total carbohydrate content and total protein content followed by a concentration of 3:2 % (vermiwash: humic acid). This study was effective in formulating an efficient chemical free fertilizer that can be further used in agriculture on a large scale.

**Key words:** *Humic acid*, Vermiwash, *Allium cepa*; plant growth parameters

Agriculture is an art, science and industry of managing the growth of plants and animals for human use. Two major types of agricultural systems are followed in India i.e., modern agriculture and traditional agriculture. Modern agriculture makes use of chemical fertilizers, pesticides and herbicides but traditional agriculture makes use of cow dung and composting materials as fertilizers for crops. Increased use of chemical fertilizers over a long period of time has led to contamination of soil that further led to the contamination of food materials. Due to this, emphasis is laid on organic farming to improve the quality of food and health of the mankind. Organic farming is a system of natural farming that employs techniques such as green manure, crop rotation, compost and biological pest control, natural fertilizers limiting the use of petrochemical fertilizers

and pesticides there by fulfilling the food and nutritional quality without depleting the natural soil system.

This situation led to the use of liquid fertilizers like vermiwash derived from vermicompost. Vermicompost in the soil favourably affects soil pH, microbial population and soil enzyme activities<sup>1</sup>. Vermicomposts are produced from organic wastes through interactions between earthworms and microorganisms, and can be utilized as plant growth media or soil amendments. Vermiwash obtained from vermicompost is found to comprise of nutrients that are easily taken up by plants and hence play an important role in the plant growth and development. They are found to possess enzymes and secretions of earthworms that would stimulate the growth of the crops<sup>2</sup>. In the recent past, studies on humic acid revealed their potential as a supplement for plant growth. This is found to increase the water holding capacity and soil fertility there by positively influencing the growth of plants.

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In view of the advantages possessed by vermiwash and humic acid, the present study tries to develop a formulation comprising these components and study their effect on the growth of the plant *Allium cepa*.

## MATERIALS AND METHOD

### Preparation of Vermiwash

Vermicompost is collected from local market in Chennai, TamilNadu, India. 100 g of vermicompost is mixed with 200 ml of water thoroughly. The mixture is filtered after one hour and the vermiwash is collected and used for further experiments.

### Preparation of Humic Acid from Lignite

Humic acid was extracted by following the procedures of International Humic Substances Society (IHSS). Crude Humic acid was used for all trials. Ten grams of coal (leonardite) was weighed and ground. It was then passed through a mesh sieve to get the fine particles and the sample was treated with 100 ml of 4% Potassium Hydroxide and mixed thoroughly. Water soluble salt of humic acid thus formed was filtered through a Whatman No. 42 filter paper to separate it from insoluble residues and 1ml of concentrated hydrochloric acid was added to bring the pH < 2. The humic acid will get precipitated at the bottom of the beaker and the precipitate thus obtained is used for further experiments.

### Formulations used for the experiment

The three categories of formulation includes

Vermiwash (1 to 10%); Humic acid (0.5% to 5%); Vermiwash and Humic acid (1:0.5, 2:1, 3:1.5, 4:2.5, 5:2.5, 6:3, 7:3.5, 8:4, 9:4.5, 10:5); and control trials were separately maintained.

### Effect of humic acid and Vermiwash on growth of *Allium cepa* seeds

The effect of the above mentioned formulations was studied on *Allium cepa*. The seeds (10) were grown in petri plates and the treatments were given as per the above mentioned concentrations. The effect of the vermiwash and humic acid on seed germination of *Allium cepa* was studied by soaking the seeds in water which served as the control in one experiment, and two other experiments where the seeds were drenched in vermiwash, humic acid and their combinations. During the growth period, the germination rate was

determined followed by finding the shoot length and root length.

### Effect of humic acid and Vermiwash on growth of *Allium cepa* in pots

Garden soil was collected from the premises of University and mixed with 10% of cow dung and filled in 25×10 cm size black color polythene bags. 1.5 kg of soil added in 40 bags and in each bag three onions were sown. After germination, the effect of humic acid and vermiwash was studied individually at different concentration followed formulated vermiwash with humic acid (1:0.5 to 10:5%). Bags were watered regularly and every 15 days monitoring plant height, no of branches and biochemical analysis such as total carbohydrates, total proteins contents were observed.

## RESULTS AND DISCUSSION

The extraordinary ability of earthworms in the formation of soil and maintaining its fertility that plays a crucial role in balancing the ecosystem is well acknowledged in the past. Vermicompost is the compost which is a finely divided material with excellent porosity, water holding capacity, aeration, drainage and structure. It is prepared using earthworms and the nutrient quality of the vermicompost depends on the input material. Evidences exist stating that vermicompost remains a good promoter for the growth of plants by improving the biological, chemical and physical properties of the soil.

Vermiwash is the liquid usually obtained after the passage of water through the vermicompost. This is used as fertilizer as this is a mixture of secretory and excretory products of the earthworm and also includes the micronutrients of the soil. Therefore it is used for the growth of the plants and also acts as mild biocide<sup>1-15</sup>.

### Effect of vermiwash on *Allium cepa* seed germination

Various concentrations of vermiwash were used to evaluate its effect on the growth of *Allium cepa* using germination rate, root length and shoot length as parameters. 100 % germination was observed in the plates with 4 %, 6 % and 7 % vermiwash. But at 4 % concentration, the root and the shoot length were found to be highest with the average to be 7.4 cm and 5.8 cm

respectively. The concentrations beyond 4 % did induce the germination, root and shoot growth but 4 % was identified to be ideal concentration for maximum growth.

Similar kind an effect was observed where

in 10 % Vermiwash was able to bring about 100 % germination in the seeds of *Hibiscus sabdariffa* and *Phaseolus vulgaris* when used along with plant growth promoter gibberellic acid<sup>3</sup>. This reports suggested the use of 10 % Vermiwash for effective

**Table 1.** Effect of various concentrations of vermiwash on the growth of *Allium cepa* seeds

Conc of vermiwash (%)	Germination rate (%)	Root length (cm)	Shoot length (cm)
1	30	6.1±0.21	3.44±0.14
2	40	6.5±0.08	3.45±0.18
3	50	5.6±0.12	5.33±0.15
4	100	7.4±0.08	5.90±0.10
5	90	6.7±0.02	4.37±0.15
6	80	6.9±0.05	4.47±0.15
7	80	5.6±0.36	5.13±0.12
8	60	5.4±0.09	5.41±0.09
9	60	6.3±0.10	5.44±0.14
10	70	5.3±0.16	5.25±0.14

**Table 2.** Effect of vermiwash on the growth of *Allium cepa* in pots

% Vermiwash	Avg no. of branches	Avg shoot length (cm)	Protein (µg/g)	Carbohydrate (µg/g)
1%	4.00	6.00	110	80
2%	7.66	11.66	165	60
3%	8.66	18.66	125	160
4%	11.00	20.33	220	260
5%	6.33	19.66	90	210
6%	6.33	19.00	165	150
7%	8.00	18.7	160	140
8%	6.66	18.00	155	110
9%	4.00	6.00	160	100
10%	6.66	14.33	150	80

**Table 3.** Effect of various concentrations of humic acid on the growth of *Allium cepa* seeds

Conc of vermiwash (%)	Germination rate (%)	Root length (cm)	Shoot length (cm)
0.5	20	0.25±0	0.65±0.07
1	20	3.3±0.14	3.3±0.14
1.5	100	3.8±0.14	5.85±0.07
2	80	3.9±0.14	5.5±0.14
2.5	80	3.35±0.35	3.35±0.07
3	80	1.45±0.48	3.35±0.35
3.5	100	3.2±0.14	3.75±0.21
4	60	2.75±0.07	2.9±0.00
4.5	40	4.5±0.14	3.25±0.21
5	20	2±0.14	1.15±0.07

seed germination but in the current study only 4 % of Vermiwash was found to be sufficient for attaining 100 % germination rate thereby stating its effectiveness in the growth of *Allium cepa*.

#### Effect of vermiwash on *Allium cepa* Pot culture

The effect of vermiwash on the growth of *Allium cepa* was also studied at the pot level where in the number of branches and shoot lengths were taken into consideration. Likewise, at this stage, the total protein and carbohydrate content was also determined to evaluate the effect of vermiwash on certain biochemical parameters and the results are provided in Table 2. From the results, it is observed that 4 % vermiwash was ideal for the growth of seeds in the pot study. At this concentration, there is more number of branches with maximum shoot length. Moreover in this

condition, there is high protein and carbohydrate content in the leaves of the plant.

Likewise, the positive effect of Vermiwash on the seeds of *Vigna munga*, *Vigna radiate* and *Sesamum indicum* was found where in there was an increase in the growth parameters like the root length, shoot length, number of twigs and leaves and total biomass<sup>4</sup>. A study conducted by Prabhu (2006) reported the use of vermiwash for the growth of seeds of cowpea and paddy crops<sup>5</sup>. The results showed an improved germination percentage and seedling vigour. This increased growth may be due to the proteases in the Vermiwash that may help in the germination and the amylases could be a simple carbon source that promotes plant growth and productivity<sup>6</sup>.

**Table 4.** Effect of humic acid on the growth of *Allium cepa* in pots

% Humic acid	Avg no. of branches	Avg shoot length (cm)	Protein ( $\mu\text{g/g}$ )	Carbohydrate ( $\mu\text{g/g}$ )
0.5	6.66	9.00	150	110
1.0	9.66	13.33	150	120
1.5	9.33	23.33	160	160
2.0	15.00	29.66	160	135
2.5	9.33	24.00	170	130
3.0	7.33	20.00	150	105
3.5	7.33	11.66	145	100
4.0	7.33	8.33	140	90
4.5	10.5	14.00	135	70
5.0	12	20.5	150	95

**Table 5.** Effect of different combinations of humic acid and vermiwash on the growth of *Allium cepa* seeds and results of pot study

Conc of humic acid and vermiwash (%)	Seed growth in plates			Avg length of branches (cm)	Pot study		
	Germination rate (%)	Root length (cm)	Shoot length (cm)		Avg length of shoot (cm)	Protein ( $\mu\text{g/g}$ )	Carbohydrate ( $\mu\text{g/g}$ )
0.5:1	70	2.70 $\pm$ 0.08	2.37 $\pm$ 0.04	6	9	110	220
1:02	90	4.10 $\pm$ 0.28	3.70 $\pm$ 0.14	5.66	6.66	165	175
1.5:3	100	6.17 $\pm$ 0.15	5.32 $\pm$ 0.08	8	11.33	125	250
2:04	60	3.59 $\pm$ 0.12	1.87 $\pm$ 0.05	8	8.33	165	175
2.5:5	60	3.40 $\pm$ 0.28	3.40 $\pm$ 0.14	3.33	2.66	90	110
3:06	50	3.00 $\pm$ 0.14	2.55 $\pm$ 0.07	5	5.66	220	100
3.5:7	40	2.65 $\pm$ 0.07	4.13 $\pm$ 0.15	3.66	2.66	210	90
4:08	30	1.55 $\pm$ 0.21	2.25 $\pm$ 0.21	7	5	150	85
4.5:9	20	2.40 $\pm$ 0.14	2.57 $\pm$ 0.24	7.33	6.33	140	75
5:10	20	1.55 $\pm$ 0.21	1.15 $\pm$ 0.07	6.33	8	140	50

### **Effect of Humic acid on *Allium cepa* seed germination**

The effect of humic acid on the growth of seeds was evaluated and the results are provided in Table 3. The results clearly indicate that humic acid (1.5 %) was ideal for the growth of *Allium cepa* seeds. At this concentration, there was 100 % germination with maximum growth of root (3.8 cm) and shoot (5.8 cm).

These results go in accordance with various other reports that assert the beneficial property of humic acid as growth enhancer in plants. A study conducted by Patil *et al.* (2010) reported that the seeds of *Triticum aestivum* (L.) cv<sup>7</sup>. Showed good response in terms of seed germination and vegetative growth when treated with humic acid. Enhanced performance of humic acid was noticed by Xue *et al.* (1994) in various crops like wheat, maize, cotton and rape seed when compared to diammonium phosphate and other chemical fertilizers<sup>8</sup>. It was also found to increase the length of the seedlings of lettuce and tomato and found to increase the nutrient content of okra<sup>9,10</sup>. In this line, Prakash *et al.* (2014) also reported that the germination rate of *Raphanus sativus* increased in the presence of humic acid<sup>11</sup>.

### **Effect of Humic Acid on *Allium cepa* in Pot Study**

Various reports suggested the use of humic acid in particular concentrations as a factor that promotes growth of the roots and aerial parts of several plants and also encouraged the absorption of nutrients. The effect of humic acid on the growth of *Allium cepa* in pot study was conducted along with the estimation of protein and carbohydrate content. In this study, it is observed that 2 % humic acid is able to bring about maximum growth of the plant where the average number of branches is 15 with significant amount of protein (160 µg/g) and carbohydrate (135 µg/g).

This increase in growth parameters could be because of the uptake of minerals that would promote the root length and further enhance the weight of the crops<sup>12-18</sup>. Humic acid plays an important role in freeing up the nutrients in the soil and making them available for the plants. They are also known to chelate micronutrients and thereby increase their bioavailability<sup>19</sup>.

### **Development of formulation with vermiwash and humic acid and its effect on the seed germination and plant growth**

Though not exactly termed as organic fertilizers, humic acid and vermiwash indirectly play a crucial role in promoting the growth of the plants and hence they act as growth supplements. The combined effect of vermiwash and humic acid in various concentrations on the growth of *Allium cepa* seeds followed by growth in pots was estimated and the results are provided in Table 5.

Different concentrations of humic acid and vermiwash were used to evaluate their combinatorial effect on the growth of *Allium cepa*. It was found that humic acid and vermiwash in the ratio 1.5% and 3%. At this concentration, the germination rate was 100 % with maximum root and shoot lengths. Lower concentrations of humic acid and vermiwash were found to be favourable for the growth of the seeds as higher concentrations tend to reduce the germination as

well as the growth parameters. Similarly the combined effect of humic acid and vermiwash was studied when the seeds were grown in pots. Important parameters like number of branches and shoot length were studied along with protein and carbohydrate content. It was found that in pot study also the ideal concentration of humic acid and vermiwash for the growth of plants was 1.5 : 3 % respectively. Hence the ideal concentration of humic acid and vermiwash for the maximum growth of *Allium cepa* was found to be 1.5 : 3 %.

Zea mays were cultivated in fields using vermiwash as biofertilizer which majorly reflected on the production of leaves with application time<sup>3</sup>. Zea mays was cultivated in fields using vermiwash as biofertilizer which majorly reflected on the production of leaves with application time<sup>20</sup>. Further attempts were successful in using vermiwash obtained from kitchen, agro and animal wastes for the growth of plants<sup>21</sup>. Earlier studies have suggested the beneficial property of humic acid on the growth of certain plants like *Spirulina plantensis*, *Pleurotus florida*, *Stevia rebaudiana* and *Morus alba* also formulated of seaweed liquid fertilizer using with humic acid and effects of *Arachis hypogaea* was reported thereby strongly advocating the suitability of humic acid to different types of plants and promoting their growth<sup>24-26</sup>.

Independently, the concentration of vermiwash and humic acid required for promoting the growth of seeds and plants was found to be 4% and 1.5 % respectively. But when used together the required concentration for maximum growth was 3 % and 1.5 % respectively. Vermiwash is considered to be a nutritious material as it contains the various secretions of earthworms including enzymes that may further stimulate the growth of plants. Likewise humic acid is known to enhance soil fertility and water holding capacity. Hence using a combination of humic acid and vermiwash would definitely improve the overall standards of the soil which further facilitates the growth of the plants<sup>22, 27</sup>.

### CONCLUSION

The present study reported the favorable effect of humic acid and vermiwash on the growth of *Allium cepa*. Three sets of experiments were carried out where in the seeds on *Allium cepa* were grown separately using humic acid and vermiwash and vermiwash and humic acid in combination in various concentrations. The ideal concentration of humic acid and vermiwash was found to be 1.5 % and 4 % independently, while 1.5:3 % was found to be the ideal concentration when used in combination. Therefore this study successfully demonstrates the use of these organic products as growth supplements for plants and suggests the possibility of using these as biofertilizers.

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