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Effect of Hydrogen Peroxide and Vermicompost on Germination and Development of *Vigna Mungo*

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Abstract: With the aim of securing the nutritional security with legumes the study aims at the effect of hydrogen peroxide and vermicompost on germination as well as morphology of *Vigna mungo*. Before sowing the seeds were treated with hydrogen peroxide as well as vermicompost. Then after sowing observations were made on the percentage of germination, plant height, number of leaves, width and height of leaves, area of leaves and root length and nodule formation. The percentage of germination is calculated after 7 days of seed sowing. The plant height was measured at 6, 12, 17, 24, 35 and 42 days after seed germinating with the help of measuring scale. The root length was measured after 16 and 25 days of germination. The leaf width and length were also measured with the help of measuring scale and leaf area was calculated on this basis. From the experiment conducted it was found that the seed germination percentage was highest in Vermicompost treated soil in *Vigna mungo*. The germination percentage in treatment with vermicompost (T3) was highest 95% then followed by 85% germination rate in treatment with hydrogen peroxide (T2) and the least 65% in the control (T1). Moreover, the seeds treated with vermicompost show tremendous increase in plant height, increase in number of leaves and relatively large leaf area compared to the seeds treated with hydrogen peroxide and then followed by control. Therefore, the study recommends the use of vermicompost over any inorganic material or chemical.

Keywords: Vermicompost • Hydrogen peroxide • Randomised complete design • Plant height • Leaf area

Introduction

Legumes belonging to the family Leguminosae, holds an important position other than cereals as sources of human food as they are an absolute source of nutritional security with cereals to tackle malnutrition in the undeveloped as well as under developed nations. They contain more protein material than any other vegetable product and so are nearer to animal flesh in food value as well as carbohydrates, fats, vitamins and minerals are also present in them in small composition. The high protein content is correlated with the presence on the roots of many legumes that contain nitrogen fixing bacteria. These bacteria are able to utilise free atmospheric nitrogen and convert it into nitrates thus, augmenting the supply of nitrogenous material available for the plants. Nearly 18,000 species of legumes are known and many are of importance as industrial, medicinal or food plants. India accounts for about 33% of world production of pulses. Since the production of the pulses is at constant rate, the net availability of the pulses reduced to 41.7g/day/capita in 2016 from 60 gm in 1951 which is much lower than the value recommended by Indian council of medical research (ICMR), which is 65 g/day/capita.

Vigna mungo, commonly known as black gram, belonging to family 'Fabaceae', is also known as urid, urd, urd bean, black matpe bean, urad dal or urad. It is extremely nutritious. It comprises of moisture (10.9%), protein (24.0%), fat (1.4%), fiber (0.9%), minerals (3.2%) and carbohydrates (59.6%). Black gram is a vital pulse crop cultivated in summer under a wide range of agro-ecological zones, mostly of the rain fed nature. It is cultivated in many South Asian countries. In India, black gram was cultivated in an area, production and productivity of 4.49 m ha, 2.92 m tons and 651 kg/ ha, respectively (Directorate of Economics and Statistics, 2016-17). Important states producing black grams are Madhya Pradesh (24.11%), Maharashtra (9.68%), Rajasthan (7.42%), Karnataka (2.62%) and Bihar (0.42%). In Karnataka, black gram occupies 71 thousand ha with a production of 20 thousand tons and productivity is 282 kg ha^{-1}. In Tamil Nadu, the production of rice fallow pulses contributes about 40-50% of the total pulses production in which black gram also contributes a major part. The rate of black gram production is not sufficient to meet the demand of the growing population in India. Thus, many agronomic practices have been initiated to boost the productivity of the crop. Germination as the first stage of the plant development is one of the critical stages in the life cycle of plants and is a key process in the emergence of the seedling (De-Villiers *et al.*, 1994). During germination, some seeds germinate while some do not due to which it is necessary to look out ways to increase the germination rate.

Hydrogen Peroxide (H_2O_2) is a signal molecule which mediates a wide range of physiological and biochemical reactions during the whole period of plant growth. Earlier, it was recognized as a virulent molecule that causes negative effects at different levels of cell organization and therefore leads to losses in the cell viability. H_2O_2 is continuously generated from various sources during the normal metabolism in the plant cells. The biological effect of hydrogen peroxide is mostly dependent on its concentration and also on the site of production. Generally, at low concentrations, it acts as a signalling molecule (He *et al* 2009) whereas at the higher concentrations, it provokes the onset of cell death (Gechev and Hille, 2005). The accumulation of the H_2O_2 and other reactive oxygen species has been distinguishing in seed biology during imbibitions and also at a point of germination (Schopfer *et al* 2001, Xin *et al* 2014, Kubala *et al* 2015).

Vermicomposting has long been recognized as a low cost and environmentally sound process for the treatment of many organic wastes such as animal manures, sewage sludge, crop residues and industrial waste (Albanell *et al* 1988, Edwards and Burrows 1988, Atiyeh *et al* 2001, Edwards *et al* 2004, Arancon *et al* 2008, Lazcano and Dominquez 2011). Vermicompost is derived from the accelerated biological degradation of organic wastes by earthworms and micro-organisms living in the vermicompost mixture. Vermicompost promotes plant growth, enhances germination and increase yield in various vegetables, field crops, forest nursery plants and ornamentals. The beneficial effect of Vermicompost was first highlighted by Darwin (1881). Vermicompost contains micro site rich in available carbon and nitrogen **(**Sudhakar *et al* 2002). Worm cast incorporated soils are also rich in water soluble P (Gratt 1970) and contained two to three times more available nutrients than surrounding soils (Sudhakar *et al* 2002) which encourages better plant growth. Bano and Kale (1987) reported that the application of the vermicompost along with other chemical fertilisers recorded higher yield of brinjal. Salvagi and Salvagi (1991) found that there is increase in the germination percentage, shoot length and dry matter of hybrid sorghum (CSH-5) upon seed treatment with vermicompost. Vermicompost suppress parasitic attacks dramatically and also have shown to increase germination rates, growth etc. in wide ranges of crops. Similar results were also reported by application of vermicompost on seed germination in mung bean by Nagavallemma *et al* (2004).

This study aims to determine the effect of H_2O_2 and vermicompost on the seed germination in *Vigna mungo* and early seedling growth. Research has been done in general and does not distinguish between the types of vermicompost.

Material and methods

The materials used in this experiment are Black gram seeds, hydrogen peroxide and vermicompost. The black gram seeds were collected from the local market. Vermicompost was collected from Agriculture department Krishi bhawan Talab tillo, Jammu and Kashmir. The experimental design used in this study was randomized complete design (RCD). The treatment consisted of 3 (three) treatment conditions.

- First treatment (T_1) is control in which black gram seeds were not treated with any material.
- In the second treatment (T_2), seeds were treated with Hydrogen peroxide concentration (10 ml H_2O_2 in 50 ml water) for time interval of 6 hours.
- The third treatment (T_3) includes combination of mixing vermicompost with soil. In this treatment 50 g of vermicompost was being added to the soil.

The seeds were then drained and planted in pots that have been filled with soil in accordance with the different treatments. The experiment of pre sowing treatment was carried out in college laboratory whereas seeds were sown in pots for germination under natural condition at home.

Soil collection and Potting

The soil was collected from the nearby garden The pebbles and other waste materials such as debris, leaves, seeds, weeds, plant roots, plastic waste etc. were removed. The soil was then taken into the respective experimental pots in equal amount. Each treatment is repeated three times, therefore, each experimental unit consists of three pots. So, the total number of pots used in the experiment is 9. The number of seeds treated for each experimental unit is 20. They are planted in each pot numbering 7, 7 and 6 for respective treatment unit. The pots were labelled before seed planting according to the respective treatment. The seeds after planting were then watered and pots were kept at open place and covered with polythene shelter to protect them from any damages.

Care and Maintenance of Plants

Maintenance includes only watering and weeding. Plantlets were watered continuously. Pots were constantly observed to check the growth of other unwanted plants such as weeds in the experimental pots. The weeds were plucked out from the pots. The plants in their initial stages of growth were protected from the extreme sun light and rain by covering them with a roof made up of polythene bags or by placing them in a protected environment. Thus, care was taken to protect the plants from extreme biotic and abiotic factors which may affect the plant's normal growth and development.

The observations were made on the percentage of germination, plant height, number of leaves, width and height of leaves, area of leaves and root length and nodule formation. The percentage of germination is calculated after 7 days of seed sowing. The plant height was measured at 6, 12, 17, 24, 35 and 42 days after seed germinating with the help of measuring scale. The root length was measured after 16 and 25 days of germination. The leaf width and length were also measured with the help of measuring scale and leaf area was calculated on this basis.

Data collection and Analysis

Data collected were based on germination percentage, trend of germination and early growth of seedlings. Total germination was obtained by visual counting of the number of germinated seedlings from the first day of seedling emergence and germination percentage was calculated. Germination percentage was expressed as follows.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds put for germination}} \times 100$$

After seven days, seedling growth attributes like seedling height, number of leaves, length and width of leaves, leaf area, root length and nodule formation were determined and recorded after an interval of seven days.

Determination of Plant growth

The growth parameters viz., plant height and leaf area was monitored to check the impact of the experimental treatments provided on the growth and development of the growing seedlings. Plant height was measured as the distance from the ground level to the point where the topmost pair of leaves forked as given by Haizel and Aheikpor (1975). Leaf area was determined by the formula given by Montgomery (1911) who first suggested the leaf area of a plant can be calculated from linear measurement of leaves using the general relationship below:

$$\mathbf{L.A = C.F \times L \times W}$$

Where, L is the length of leaf, W is the width of leaf And C.F is the coefficient factor

The coefficient factor for *Vigna mungo* is 0.6124 as calculated by Mishra, Bera and Sasmal (2000). The plant height, leaf length and leaf width were determined using a meter rule (in cm). The leaf growth and leaf area were determined after every seven days. To determine the plant growth, height of plant was recorded after every seven days. The leaf area was also recorded after every seven days. The number of leaves in each pot was also counted visually along with these parameters. The root length was calculated using meter scale (in cm). The roots were also observed for checking the nodule formation in them. The record of the nodule formation was also kept along with the root length. The root length was calculated after every 28 days of the germination of the seedlings.

Results

The pre-germination treatment given to black gram seeds with hydrogen peroxide and vermicompost provided the following results:-

Plant type: Erect habit, incompletely dominant over spreading over spreading habit, single gene pair.

Leaf type:

1) Monogenic, ovate dominant over lanceolate
2) Hastate leaf shape dominant over ovate, controlled by duplicate dominant genes

Pod pubscence: pubescent pod monogenically dominant over non-pubescent type.

Pod color: usually monogenic and black color dominant over straw or brown

Seed coat color:

1) Monogenic, green dominant over brown
2) Black coat, monogenic, dominant over brown
3) Shining seed coat, monogenic, dominant over dull

From the experiment conducted it is observed that the seeds treated with vermicompost shows significant increase in plant height; number of leaves and relatively large leaf area compared to the seeds treated with hydrogen peroxide followed by control. Fig. 1 to 9 illustrates the many phases of plant growth across several treatments, from germination to biomass (leaf, stem, and root) development. It was found that, the seed germination percentage was highest in Vermicompost treated soil in *Vigna mungo*. High seed germination was observed in vermicompost treated soil.

The germination percentage in treatment with vermicompost (T3) was highest 95% followed by 85% germination rate in treatment with hydrogen peroxide (T2) and the least 65% in the control (T1) (Table 1).

I) Germination stages

Control **A** Control **B** Control **C**

Control **A** Control **B** Control **C**

Figure 1. Seedling and germination of *Vigna mungo* in control pots

Figure 2. Seedling and germination of *Vigna mungo* in hydrogen peroxide treated pots

Figure 3. Seedling and germination of *Vigna mungo* in Vermicompost treated pots

II) Stem

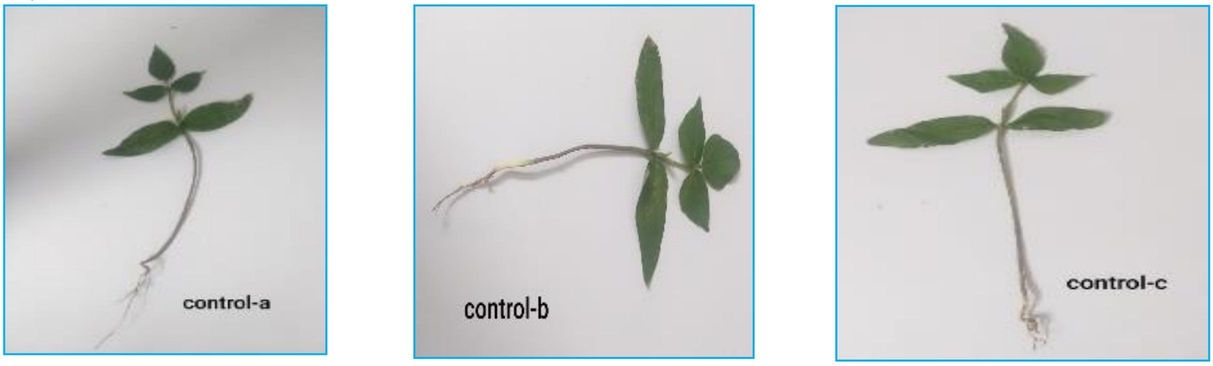

Figure 4. Stem height in control pots

Figure 5. Stem height in Hydrogen peroxide treated pots

Figure 6. Stem height in Vermicompost treated pots

III) Root length

Figure 7. Root length in control pots

Figure 8. Root length in hydrogen peroxide treated pots

Figure 9. Root length in Vermicompost treated pots

A) Germination studies

Table 1. Effectiveness of pre-germination treatments on the seeds of *Vigna mungo*

Pre-germination treatment	Number of seeds treated	Number of seed germinated	Germination %
T1	20	13	65
T2	20	17	85
T3	20	19	95

T1 - Control, T2 - Hydrogen peroxide, T3 – Vermicompost

The germination percentage in treatment with vermicompost was highest 95% then followed by 85% germination rate in treatment with hydrogen peroxide and the least germination rate was recorded 65% in the control. The highest germination rate was recorded in plants given treatment with vermicompost as compared to other treatments.

Table 2 presents a comparison of plant height, leaf count, leaf surface area, and root length amongst various treatments. The plant height observed in vermicompost treated plants (T1) was highest with maximum average height of 19.42 cm, large number of leaves (34) with maximum leaf area (15.56 cm^2). It was followed by the plants treated with Hydrogen peroxide (T2) showing the average maximum height (20.03 cm) with large number of leaves (27) along with the leaf area of 15.91 cm^2. The least number of values were reported in control (T3) having the maximum height of 17.57cm with maximum number of leaves obtained (18) along with leaf area of about 15.18 cm^2.

Table 2. Effect of Control, Hydrogen peroxide and Vermicompost on plant growth and development in *Vigna mungo*.

Treatment	Crop age (in days)	Plant height (in cm)	Number of leaves	Leaf area (cm^2)	Root length (in cm)
T1 – Control	1	6.82	2	4.27	4
	2	7.97	2	6.24	5.3
	3	10.92	5	7.55	6.5
	4	12.63	8	9.62	7.2
	5	14.45	12	10.68	8.6
	6	16.15	15	13.00	9.4
	7	17.57	18	15.18	10.7
T2 – Hydrogen peroxide	1	4.84	2	4.02	4.3
	2	6.45	2	6.00	6.6
	3	12.88	5	7.95	8.2
	4	15.38	9	12.70	10.4
	5	16.85	15	13.13	12.5
	6	18.36	21	13.60	14.1
	7	20.03	27	15.91	16.3
T3 - Vermicompost	1	5.20	2	4.29	9.1
	2	7.12	2	7.20	10.6
	3	12.9	5	8.65	11.8
	4	15.66	11	11.83	12.7
	5	16.45	19	12.76	13.9
	6	17.99	28	14.49	14.6
	7	19.42	34	15.56	15.8

Crop age - calculated after seven days, Root length - calculated after 10 days

The maximum number of leaves was observed in plants grown in soil treated with vermicompost (T3) i.e. maximum value of 34. The largest maximum leaf area of about 15.91 cm^2 was noted in plants treated with hydrogen peroxide along with the maximum height of 20.03 cm obtained. The maximum root length was noted in vermicompost (10.6 cm), followed by plants treated with hydrogen peroxide (6.6 cm) and the least one observed in control i.e. 5.3 cm length of root. The seedlings treated with vermicompost show comparatively higher root length as compared to the other treatments. The seedlings grown as a result of pre-germination treatment with vermicompost has highest number of leaves and the seedlings treated with hydrogen peroxide showed maximum average height as well as comparatively large leaf area compared to the other treatment.

Discussion

To ascertain the effects of these three treatments—hydrogen peroxide, vermicompost, and control; vermicompost treated seeds show a notable increase in plant height, number of leaves, and relative leaf area when compared to seeds treated with hydrogen peroxide followed by control. It was found that, the seed germination percentage was highest in Vermicompost treated soil in *Vigna mungo*. High seed germination was observed in vermicompost treated soil. Both the treatments, T_2 (Hydrogen peroxide), T_3 (Vermicompost) supported the total seed germination when compared with T_1 (control). The rate of germination was found to be highest in the treatment with vermicompost i.e., 90% then followed by treatment with the hydrogen peroxide i.e. 85% when compared to the germination rate in the control. In a study conducted by Van Groenigen *et al.* (2014), it was found that the addition of vermicompost significantly increased shoot biomass by 78% and root biomass by 57%. Hydrogen peroxide has been used largely as a germination stimulant. These findings are consistence with the finding of Zeinalabedini *et al* (2009); Katzman *et al* (2001); Ogawa and Iwabuchi (2001); Chen and Lin (1994); Fontaine *et al* (1994), when exogenous application of hydrogen peroxide improved seed germination in many plants, including camphor, barley, spinach, *Zinnia elegans* and almond. The seedlings treated with vermicompost show significant rise in plant length, number of leaves, leaf surface area as well as root length.

The plant height observed in vermicompost treated plants (T_1) was highest with maximum average height achieved 19.42 cm, large number of leaves (34) with maximum leaf area (15.56 cm^2). Similar results were reported by Deng *et al* (2012) showing hydrogen peroxide positively influences root growth and metabolism in leaves of sweet potato when applied exogenously. H_2O_2 could promote the formation and growth of adventitious roots of seedling explants in cucumber. Vermicompost has also been reported to improve the yield parameters of wheat (Yousefi *et al* 2014), maize (Kmetova and Kovacik 2014) tomatoes (Zucco *et al* 2015) and peppermint (Ayyobi *et al* 2014). The seedlings treated with vermicompost show comparatively higher root length as compared to the other treatments. The seedlings grown as a result of pre-germination treatment with vermicompost has highest number of leaves and the seedlings treated with hydrogen peroxide showed maximum average height as well as comparatively large leaf area compared to the other treatments.

Conclusion

Current study suggests that the pre-treatment of seeds with vermicompost and hydrogen peroxide have tremendous impact on plant biomass (i.e. height, number of leaves, and leaf area). The highest plant length and root length was recorded in plants given pre-germination treatment with hydrogen peroxide. The maximum number of leaves was recorded in vermicompost treatment as compared to other treatments. The highest germination rate was recorded in plants given treatment with vermicompost as compared to other treatments. The pre-germination treated seedlings exhibit a higher rate of germination, more leaves, more leaf area, and longer roots than the control group. These results offer a novel approach for the next generation of seedlings. Moreover, they are highly interesting, especially in the context of boosting agricultural output in developing and commercial applications by vigorous seed treatments.

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