Seed germination performance in *Trigonella foenum-graecum* L. under the influence of Gibberellic acid, Oxygenated Peptone and Vermiwash

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**ABSTRACT**

Oxygenated peptones and Vermiwash are commonly used in organic farming practices. In the present investigation, experiments were carried out to study the influence of Gibberellic acid (50 ppm), Oxygenated peptone (1% aqueous) and Vermiwash (20%) on fenugreek (*Trigonella foenum-graecum* L.) var. early bunching during seed germination by using petri-plate method. Distilled water was considered as control. Oxygenated peptone and Vermiwash showed promontory effect on germination percentage. All three treatments stimulated vigour index and also showed enhancement in fresh and dry weights of root and shoot. Oxygenated peptone and Vermiwash showed supremacy in root length while GA treatment remarkably enhanced shoot length. Oxygenated peptone, Vermiwash and GA treatments showed significant improvement in the amount of total carbohydrates, soluble proteins and activities of enzymes like amylase, protease and catalase. The study revealed that Vermiwash, Oxygenated peptone and GA improve the process of germination with positive alteration in biochemical aspects.

**Keywords**: Organic farming, Oxygenated peptone, Vermiwash, Gibberellic acid.

**INTRODUCTION**

In the modern era of agriculture, organic farming is an urgent need to reclaim deteriorated agricultural land for sustainable agriculture. Organic farming is productive systems which take
care of soil and rely in use of organic fertilizers, crop rotation, biocontrol, phytoremediation, vermintechnology and biofertilizers while strongly deny synthetic agrochemicals, plant growth regulators and genetically modified organisms. Werner (1997) and Tester (1999) suggested organic fertility inputs, such as composted farm yard and green manure, improve the soil physical properties by lowering bulk density, increasing water-holding capacity and improving infiltration rates. Organic farming systems are more sustain- able than conventional agricultural techniques and represent commercially viable alternative (Marangoni et al., 2004). Pretty et al., 2002), observed that implementation of organic farming in some developing countries causes higher yield of production. Among many benefits of organic farming, the most obvious advantage that might mostly be considered for small-scale farmers is their traditional knowledge of natural environment can be well matched with organic farming (Closter et al. 2004).

Earthworms play significant roles in agro-ecosystems. Their feeding and burrowing activities incorporate organic residues and amendments into soil, enhancing decomposition, humus formation, nutrient cycling and soil structural development (Mackay & Kladivko, 1985; Kladivko et al., 1986). Vermicompost and Vermiwash are crucially important in organic farming. Vermicompost are typically finely divided peat like materials with high porocity, moisture content, aeration and water holding capacity (Edwards and Burrows, 1988). Vermiwash is a liquid manure obtained from earthworms while vermicompost preparation. Apart from nitrogen, potash, phosphourus, other micronutrients it also contains phytohormones like auxins and cytokinins. Vermiwash contain nitrogen fixing bacteria such as Azotobacter sp., Agrobacterium sp., and Rhizobium sp. and also enzyme cocktail of proteases, amylases, urease and phosphatase (Zambare et al., 2008). Oxygenated peptone is organic, creamy white powder. It consists of 100 mg/g oxygen, 650 mg/g peptone & 250 mg/g inert filler compound. It is a source of soluble nitrogen as well as oxygen. Organic substance like oxygenated peptone can be successfully used for soil conditioning under organic farming (Patil et al., 2008; Thakare et al., 2011; Jagtap, 2012).

Fenugreek (Trigonella foenum-graecum L.), a member of Fabaceae is an annual herb with trifoliate leaves, cultivated worldwide as leafy vegetable, spice, forage and medicinally potential crop. It is extensively cultivated in most regions of the world for its medicinal value (Petropoulos, 2002). Fenugreek seeds are good source of protein, fat, minerals and dietary fiber (Kochar et al., 2006). Seeds are used in colic, flatulence, dysentery, diarrhea, dyspepsia with loss of appetite, diarrhea in puerperal women, chronic cough, dropsy, and enlargement of the liver and spleen (Nadkarni, 2005). The seed extracts normalize the enhanced lipid peroxidation and relieve oxidative stress by providing antioxidants in diabetic rats (Anuradha and Ravikumar, 2001). The notable chemical constituents of fenugreek are proteins that are rich in lysine and tryptophan, flavonoids such as quercetin, trigonelline, saponin, phytic acid and polyphenols (Billaudand Adrian, 2001).

Germination is resumption of plant life from seed. The phenomenon is preceded by the absorption of water by the dry seeds followed by degradation and mobilization of reserves accumulated during seed formation and maturation. Germination percentage, speed of germination and germination performance at morphological and biochemical level greatly determines the yield of crop. Oxygen supply is most critical and fundamental requirement for growth, productivity of plants and the rate of germination increases with oxygen supply (Taiz and Zeiger, 2002). To encourage organic farming, attempts were made to study the effect Vermiwash, Oxygenated peptone and Gibberellic acid on fenugreek during germination of seeds.

MATERIALS AND METHODS

Eudrilus eugineae (Fig.1 and Fig. 2). a species of earthworm was cultured for the preparation
Seeds of fenugreek (Trigonella foenum-graecum L. var. early bunching) were surface sterilized with 0.05 % HgCl2 for minute and rinsed thoroughly with tap water and then with distilled water. The surface sterilized seeds were arranged in petri plates (10 seeds/plate) containing germination paper. 10 ml of distilled water (control), 20% Vermiwash, 1% aqueous Oxygenated peptone and 50 ppm Gibberellic acid was poured in the respective petri-dishes. Numbers of seeds germinated were recorded daily. Germination parameters like germination percentage, shoot length, root length, shoot/root ratio, fresh weight and dry weight were studied on 6 DAS using routine method. Vigour Index (VI) was calculated according to the method suggested by Baki and Anderson (1993). Biochemical constituents were analyzed on 8 DAS using methods proposed by Lowry et al. (1951) for soluble proteins and Sadasivam and Manickam (2005) for total carbohydrates. The enzyme activity was studied on 10 DAS. The enzyme activity of Amylase (E.C. 3.2.1.1) and Catalase (E.C. 1.11.1.6) was scored by the methods of Sadasivam and Manickam and that of Protease (E.C. 3.4.2.2) by the method of Penner and Ashton (1967).

RESULTS AND DISCUSSION

The treatment of GA, Vermiwash and Oxygenated peptone showed enhancing effect on morphological parameters of germinating seeds of fenugreek. Table 1 exhibit the effect GA and vermiwash and oxygenated peptone on morphological parameters of germinating seeds of fenugreek 6 days after sowing (DAS). Seeds showed 98 % germination under Oxygenated peptone treatment and control (distilled water) while 100 % under GA and vermiwash treatments. Vermiwash contain higher amount of potassium (K) than sodium (Na) (Shlrene et al., 2012). Potassium, as one of the essential macro-nutrients, is needed in higher amount for better plant growth (Bumb and Hammond, 2005). Patil, et al. (2008) reported enhancing effect of Oxygenated peptone during germination processes in Solanaceous fruit vegetables like tomato, brinjal and chilli by seed priming treatment. The effect of GA on stem elongation and leaf enlargement is well known. Riley J. M. (1987) stated that GA plays direct role in stem elongation. All three treatments promoted morphological parameters like shoot length, root length, fresh and dry weights of stem and root. GA (25.61%) showed supremacy over Vermiwash (14.03%) and Oxygenated peptone (9.29%) for shoot elongation, while Vermiwash (51.63%) and Oxygenated peptone (29.79%) showed upper hand on root elongation than control and GA.
(12.24%). Cherif et al. (1997) were advocated that oxygenated peptone supports the development of root system than that of shoot system during germination by supplying soluble nitrogen and oxygen so that seedling is established with extended root system. Due to elongation of shoot, shoot/root ratio was positively improved by GA (18.10%), while Vermiwash (-25%) and Oxygenated peptone (-16.37%) exhibited negative values because of root elongation. All treatments showed increasing effect on fresh as well as dry weights of shoot and root. GA showed upper hand in fresh weight of shoot (40%) than Vermiwash (30.05%) and Oxygenated peptone (29.18%). Dry weight of shoot was improver more by Vermiwash (60.71%) and Oxygenated peptone (44.04%) than GA (17.85%).

![Fig. 3. Biochemical constituents](image1.png)

![Fig. 4. Enzyme activity](image2.png)

**Table 1.** Effect of GA, Vermiwash and Oxygenated peptone on seeds of Fenugreek (*Trigonella foenum-graecum* L. var. early bunching) on morphological parameters of seedlings (6 DAS).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Gibberellic acid</th>
<th>Increase (%)</th>
<th>Vermiwash</th>
<th>Increase (%)</th>
<th>Oxygenated peptone</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination percentage</td>
<td>98</td>
<td>100</td>
<td>2.00</td>
<td>100</td>
<td>2.00</td>
<td>98</td>
<td>--</td>
</tr>
<tr>
<td>Shoot length (cm)</td>
<td>5.70±0.31</td>
<td>7.16±0.15</td>
<td>25.61</td>
<td>6.50±0.54</td>
<td>14.03</td>
<td>6.23±0.15</td>
<td>9.29</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>4.90±0.54</td>
<td>5.20±0.62</td>
<td>12.24</td>
<td>7.43±0.55</td>
<td>51.63</td>
<td>6.36±0.48</td>
<td>29.79</td>
</tr>
<tr>
<td>Shoot/Root Ratio</td>
<td>1.16±0.17</td>
<td>1.37±0.15</td>
<td>18.10</td>
<td>0.87±0.01</td>
<td>-25.00</td>
<td>0.97±0.01</td>
<td>-16.37</td>
</tr>
<tr>
<td>Shoot : Fresh wt. (g)</td>
<td>0.915±0.075</td>
<td>1.281±0.044</td>
<td>40.00</td>
<td>1.190±0.006</td>
<td>30.05</td>
<td>1.182±0.075</td>
<td>29.18</td>
</tr>
<tr>
<td>Root : Fresh wt. (g)</td>
<td>0.320±0.006</td>
<td>0.387±0.005</td>
<td>20.93</td>
<td>0.458±0.006</td>
<td>43.12</td>
<td>0.438±0.006</td>
<td>36.87</td>
</tr>
<tr>
<td>Shoot : Dry wt. (g)</td>
<td>0.084±0.002</td>
<td>0.099±0.007</td>
<td>17.85</td>
<td>0.135±0.003</td>
<td>60.71</td>
<td>0.121±0.006</td>
<td>44.04</td>
</tr>
<tr>
<td>Root : Dry wt. (g)</td>
<td>0.021±0.006</td>
<td>0.024±0.002</td>
<td>14.28</td>
<td>0.031±0.003</td>
<td>47.61</td>
<td>0.030±0.003</td>
<td>42.85</td>
</tr>
<tr>
<td>Vigour Index (VI)</td>
<td>1038.8±27.07</td>
<td>1236±35.01</td>
<td>21.58</td>
<td>1393±20.65</td>
<td>34.09</td>
<td>1233.8±20.01</td>
<td>18.77</td>
</tr>
</tbody>
</table>

▲ Each value is measure of mean±SD of triplicate.
Table 2. Effect of GA, Vermiwash and Oxygenated peptone on seeds of Fenugreek (*Trigonella foenum-graecum* L. var. early bunching) on biochemical constituents (8 DAS) and enzyme activity (10 DAS).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Treatments</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distilled water</td>
<td>Gibberellic acid</td>
<td>Increase (%)</td>
<td>Vermiwash</td>
<td>Increase (%)</td>
<td>Oxygenated peptone</td>
</tr>
<tr>
<td>Total carbohydrates (mg/g FW)</td>
<td>44.9 ± 6.90</td>
<td>51.8 ± 2.85</td>
<td>15.23</td>
<td>64.00 ± 3.85</td>
<td>42.53</td>
<td>61.3 ± 2.46</td>
</tr>
<tr>
<td>Soluble Proteins (mg/g FW)</td>
<td>12.052 ± 0.21</td>
<td>14.043 ± 0.25</td>
<td>16.52</td>
<td>13.268 ± 0.20</td>
<td>10.08</td>
<td>14.100 ± 0.21</td>
</tr>
<tr>
<td>Enzyme activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylase (mg maltose/5min/g FW)</td>
<td>0.70 ± 0.012</td>
<td>0.88 ± 0.008</td>
<td>25.71</td>
<td>1.08 ± 0.016</td>
<td>54.28</td>
<td>1.12 ± 0.035</td>
</tr>
<tr>
<td>Catalase (μ mole H₂O₂/min/g FW)</td>
<td>2.290 ± 0.021</td>
<td>5.146 ± 0.022</td>
<td>124.71</td>
<td>7.042 ± 0.011</td>
<td>207.51</td>
<td>7.116 ± 0.060</td>
</tr>
<tr>
<td>Protease (μg tyrosine/hr/mg protein)</td>
<td>0.112 ± 0.001</td>
<td>0.180 ± 0.002</td>
<td>60.71</td>
<td>0.15 ± 0.004</td>
<td>33.92</td>
<td>0.205 ± 0.015</td>
</tr>
</tbody>
</table>

Each value is measure of mean±SD of triplicate.

Vermiwash and Oxygenated peptone exhibited superiority in fresh weight (43.12% and 36.87% respectively) and dry weight (47.61% and 42.85% respectively) of roots than GA (Fresh weight 20.93% and Dry weight 14.28%). Vigour Index (VI) is a product of root length, shoot length and germination percentage and determines the effect external agents on seed germination. There was significant increase in VI values under the treatment of Vermiwash (1393) and Oxygenated peptone (1233.8) than that of GA (1236) and control (1038.8).

The effect treatment of GA, Vermiwash and oxygenated peptone on biochemical constituents (Fig. 3) and enzyme activity (Fig. 4) on 8DAS and 10DAS of germinating seeds of fenugreek (*Trigonella foenum-graecum* L. var. early bunching) is exhibited in Table 2. All treatments showed remarkable increase in total carbohydrates and soluble protein contents. The ascending order for total carbohydrates was Control (44.9 mg/gram fresh tissue) < GA (51.8 mg/gram fresh tissue) < Oxygenated peptone (61.3 mg/gram fresh tissue) < Vermiwash (64.0 mg/gram fresh tissue). Thakare *et al.* (2011), stated that oxygenated peptone provides soluble nitrogen along with oxygen, hence increase amount of soluble proteins significantly. The observed increasing order of soluble proteins content was, Control (12.052 mg/gram fresh tissue) < Vermiwash (13.268 mg/gram fresh tissue) < GA (14.043 mg/gram fresh tissue) < Oxygenated peptone (14.100 mg/gram fresh tissue).

In the investigation activity of enzymes like amylases, catalase and proteases increased under all treatments as compare to control. Thakare *et al.* (2011) reported that pre-sowing treatment of
oxygenated peptone and GA improves physiological performance of chick pea seed during germination. Enzymes are activated with an accompanying mobilization of reserve material ending in transport of reserve material in the embryo in somatic conditioning and thus stronger seedlings are obtained as a result of embryo growth (Muhyaddin and Wiebe, 1989). Superoxide dismutase (SOD) and catalase (CAT) acts as scavengers of reactive oxygen species which are produced during environmental stresses like high/low temperature, water stress, air pollution, UV-light and chemicals. Sangeetha (2010), in fenugreek, reported SOD and CAT increases significantly under the influence of carbendazim. The activity of catalase was significantly higher under Oxygenated peptone treatment (7.116 μ mole H$_2$O$_2$/min/g FW) followed by Vermiwash (7.042 μ mole H$_2$O$_2$/min/g FW), GA (5.146 μ mole H$_2$O$_2$/min/g FW) and control (2.290 μ mole H$_2$O$_2$/min/g FW). Oxygenated peptone showed supremacy in activity of amylase and protease over Vermiwash, GA and control. The observed order for amylase was Oxygenated peptone (1.12 mg maltose/5min/g FW) > Vermiwash (1.08 mg maltose/5min/g FW) > GA (0.88 mg maltose/5min/g FW) > control (0.70 mg maltose/5min/g FW) and for protease, Oxygenated peptone (0.205 μg tyrosine/hr/mg protein) > GA (0.180 μg tyrosine/hr/mg protein) > Vermiwash (0.150 μg tyrosine/hr/mg protein) > control (0.112 μg tyrosine/hr/mg protein).

**CONCLUSION**

In the present investigation Vermiwash and Oxygenated peptone treatments showed supremacy in biochemical aspects such as total carbohydrates, soluble protein contents as well as enzyme activities over GA treatment and control which is significant. All treatments showed enhancing effect on morphological parameters like root length, shoot length, root dry and fresh weight, shoot fresh and dry weight, vigour index. Seed response during germination crucially determines crop productivity. GA is well known growth promoting phytohormone, it enters in the metabolic pathways and alters them and hence its use is denied in the practices of organic farming. On the contrary Vermiwash and Oxygenated peptone are biological in origin and provide eco-friendly environment for luxurious growth plants, improve health of soil and increase population of beneficial microbes, hence recommended for organic farming. To study the effect of Vermiwash and Oxygenated peptone on microbial population needs further investigation.

**REFERENCES**


