RESEARCH ARTICLE

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

Jagtap DK[1*], Kashid LM[1] and Jakhi PS[2]

[1]Vidya Pratishthan's Arts, Science and Commerce College, Vidyanagari, Baramati, Dist- Pune, MS, India

[2]Depratment of Botany, Govt. Institute of Science, Nagpur, MS, India

*Corresponding author E-mail: jdattak@gmail.com

Manuscript details:

Received: 10.08.2015
Revised : 18.08.2015
Accepted: 02.09.2015
Published : 10.10.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Jagtap DK, Kashid LM and Jakhi PS (2015) Seed germination performance in *Trigonella foenum - graecum* L. under the influence of Gibberellic acid, Oxygenated Peptone and Vermi- wash, *Int. J. of Life Sciences*, 3(3): 225-231.

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 biofertlizers 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 commerce-ally 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 environ- ment can be well matched with organic farming (Closter *et. al.* 2004).

Earthworms play significant roles in agro-ecosys-tems. Their feeding and burrowing activities incor-rporate 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 prepara-tion. Apart from nitrogen, potash, phosphourus, other micronutrients it also contains phytohor-mones like auxins and cytokinins. Vermiwash contain nitrogen fixing bacteria such as Azotoba-cter 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 prot ein, fat, minerals and dietary fiber (Kochar *et al.*, 2006). Seeds are used in colic, flatulence, dysen-tery, 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 oxid-etive stress by providing antioxidants in diabetic rats (Anuradhaand Ravikumar, 2001). The notab-le 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 degrada-tion and mobilization of reserves accumulated during seed formation and maturation. Germina-tion percentage, speed of germination and germination performance at morphological and biochemical level greatly determines the yield of crop. Oxygen supply is most critical and funda-mental 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

vermiwash Large plastic container with a tap attached at the bottom was used for vermiwash draining. At the bottom of container a base layer of gravel was made which then covered by a layer of 4-5 cm thick layer of coarse sand. Partially decomposed compost with leaf litter was placed on the top of sand and earthworms were introduced. Moisture level was maintained by sprinkling water once in 2 or 3 days as per need. Vermiwash was collected after 10 day.

Fig. 1:

Fig. 2:

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%).

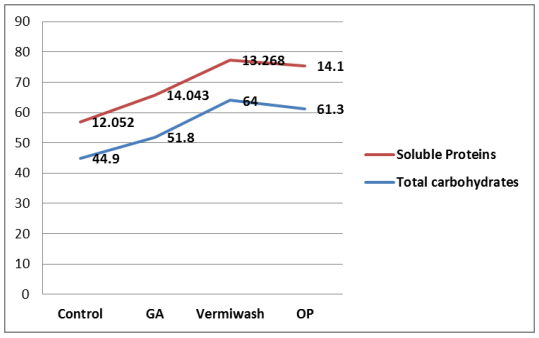

Fig. 3. Biochemical constituents

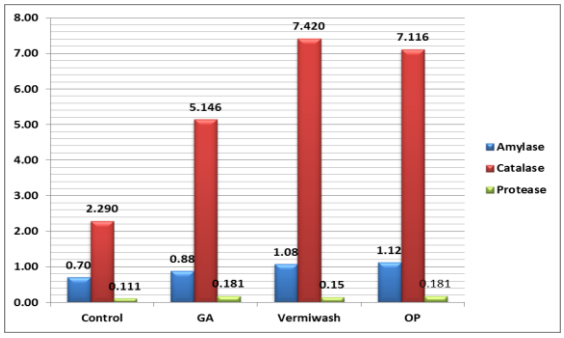

Fig. 4. Enzyme activity

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).

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

♣ 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).

Parameters	Control	Treatments					
	Distilled water	Gibberellic acid	Increase (%)	Vermiwash	Increase (%)	Oxygenated peptone	Increase (%)
Biochemical constituents							
Total carbohydrates (mg/g FW)	44.9 ± 6.90	51.8 ± 2.85	15.23	64.00 ± 3.85	42.53	61.3 ± 2.46	36.52
Soluble Proteins (mg/g FW)	12.052 ± 0.21	14.043 ± 0.25	16.52	13.268 ± 0.20	10.08	14.100 ± 0.21	16.99
Enzyme activity							
Amylase (mg maltose/ 5min/g FW)	0.70 ± 0.012	0.88 ± 0.008	25.71	1.08 ± 0.016	54.28	1.12 ± 0.035	60.00
Catalase (µ mole H_2O_2/ min/g FW)	2.290 ± 0.021	5.146 ± 0.022	124.71	7.042 ± 0.011	207.51	7.116 ± 0.060	210.74
Protease (µg tyrosine/ hr/mg protein)	0.112 ± 0.001	0.180 ± 0.002	60.71	0.15 ± 0.004	33.92	0.205 ± 0.015	83.03

♣ 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_2O_2/min/g FW) followed by Vermiwash (7.042 µ mole H_2O_2/min/g FW), GA (5.146 µ mole H_2O_2/min/g FW) and control (2.290 µ mole H_2O_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

Anuradha CV and Ravikumar P (2001) Restoration of tissue antioxidants by fenugreek seeds (*Trigonella foenum-greacum*) in alloxan-diabetic rats. *Indian J. Physiol Pharmacol.,* 45: 408-20.

Baki A and Anderson JD (1993) Vigour determination in soybean seed by multiple criteria. *Crop Science*, 3: 630-663.

Billaud C and Adrian J (2001) Composition, nutritional value and physiological properties, Science Des Ailments. 21: 3-26.

Bumb BL and Hammond LL (2005) Encyclopedia of Soil Science. 2nd Ed. (Rattan Lal CRC Press).

Cherif M, Tirilly Y and Belanger RR (1997) Effect of oxygen concentration on plant growth, lipid peroxidation and receptivity of tomato roots to *Pythium* F 707 under hydroponic conditions. *European Journal of Plant Pathology,* 103: 225-264. doi: 10.1023/A:1008691226213.

Closter AM, Embree E, Petersen M, Pedersen SH (2004) Organic farming and genetically modified crops in relation to food security. Henning Hegh Jensen Publication.

Edwasrds CA and Burrows (1988) The potential of earthworm compost as plant groth media. In: Edwards, C.A. and E. Neuhauser (Eds). Earthworms in waste and environmental management. SPB academic Press. The Hague; *The Netherlands*, pp. 21-32.

Jagtap D (2012) Physiological response of fenugreek (*Trigonella foenum-graecum* L.)

during germination under the influence of Gibberellic acid and Oxygenated peptone. *Bionano Frontier*. 5(2-II): 86-88.

Kladivko EJ, Mackay AD and Bradford JM (1986) Earthworms as a factor in the reduction of soil crusting. Soil Sci. Soc. Am. J. 50: 191-196.

Kochar A, Nagi M and Sachdeva R (2006) Proximate composition, available carbohydrates, dietary fibre and antinutritional factors of selected traditional medicinal plants. *J. Hum. Eco*. 19: 195-9.

Lowry OH, Rosebrough NT, Farr AL and Randall RJ (1951) Protein measurement with folin phenol reagent. *Journal of Biological Chemistry*, 193: 265-275.

Mackay AD and Kladivko EJ (1985) Earthworms and rate of breakdown of soybean and maize residues in soil. *Soil Biol. Biochem*. 17(6): 851-857.

Marangoni B, Rombola AD and Sorrenti G (2004) Use of natural compounds for plant nutrition and protection in organic farmed orchards. Bulletin-OILB/SROP. 27(5): 123-136.

Muhyaddin T and Wiebe HJ (1989) Effect of seed treatments with polyethylene glycol (PEG) on emergence of vegetable crops. *Seed Science Technology*. 17: 49-56.

Nadkarni AK, (2005) Indian Materia medica, Popular Prakashan Pvt. Ltd. Mumbai, India Vol. I: 1240-1243.

Patil NA, Chitale RD and Dhumal KN (2008) Role of oxygenated peptone in enhancing germination of tomato, brinjal and chilli. *Indian Journal of Plant Physiology*. 13: 137-142.

Penner D and Ashton FM (1967) Hormonal control of protease activity in squash cotyledon. Plant Physiology. 42: 791-796.

Petropoulos GA (2002) Fenugreek – The genus *Trigonella,* Taylor and Francis, London and New York.

Pretty JN, Morrison JIL, Hine RE (2002) Reducing food poverty by increasing agricultural sustainability in developing countries. *Agric Ecosyst Environ*. 95: 217-34.

Riley JM (1987) Gibberellic acid for fruit set and germination. *CRFG Journal*, 19: 10-12.

Sadasivam S and Manickam A (2005) Biochemical methods. 2nd Edition, New age International, New Delhi.

Sangeetha S (2010) Activity of superoxide dismutase and catalase in fenugreek (*Trigonella foenum-graecum*) in response to carbendazim. *Indiand J. Pharm. Sci*., 72(1): 116-118.

Shlrene Q, Embradiri A, Fatemeh P and Rupani MHI (2012) Effect of vermiwash and vermicomosting leachates in hydroponics culture of Indian Borage (*Plectranthus ambionicus*) Plantlets : UMT 11th International annual symposium on sustainability science and management, Terengganu, Malaysia. 210-214.

Taiz L and Zeiger E (2002) Plant Physiology. 3rd Edition, Prima Publishing Corporation, New Delhi.

Tester FC (1990) Organic amendment effects on physical and chemical properties of a sandy soil. *Soil Sci. Soc. Am. J*., 54: 827-831.

Thakare U, Patil N and Malpathak N (2011) Performance of chickpea under the influence of gibberellic acid and oxygenated peptone during germination. *Advances in Biosciences and Biotechnology*. 2 (1): 40-45.

Werner M (1997) Soil quality characteristics during conversion to orchard management. *Appl. Soil. Ecol.* 5: 151-167.

Zambare VP, Padul MV, Yadav AA and Shete TB (2008) Vermiwash: Biochemical and microbial approach as ecofriendly soil conditioner. *APRN Journal of Agri. and Biol. Sci*., 3(4): 1-5.