ISSN 2349-7750

INDO AMERICAN JOURNAL OF

PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

EFFECT OF DIFFERENT FORMS OF VERMIWASH ON SEED GERMINATION AND GROWTH RESPONSE IN FENUGREEK SEEDS (*TRIGONELLA FOENUM-GRAECUM)*

S Senthilmurugan

[1]Department of Zoology, Annamalai University, Tamilnadu,608 002, India

Email Id: senthilmuruganphd@yahoo.co.in

Abstract:

Earthworms are macro invertebrate and have been widely used as therapeutic drugs for thousands of years. Earthworms play a vital role in plant growth. The application of vermiwash is an ecofriendly method to increase the crop production. The root growth, number of root development, shoot growth and dry matter production of seedlings (*Trigonella foenum-graecum*) were evaluated by using different types of vermiwash (heat stress vermiwash I (0.5%) and II (1%); cold stress vermiwash I (0.5%) and II (1%). The different types of vermiwash were collected from single species (*E.foetidai*) using two methods. Vermiwash I was the body fluid of earthworms collected by using heat stress conditions and vermiwash II was the cold stress conditions collected after passage through the column of the worm action. The ten replicates of two days old seedlings were assembled in five treatments. The root growth and numbers of roots development of seedlings were significantly superior in vermiwash I and II than other treatments. The maximum drymatter of seedling was in ver I but the mean shoot height of seedling was maximum in ver II (1%). Therefore ver IIL was superior in seedlings growth to other types of vermiwash and all types of vermiwash performed compare to control. The growth inducing substance and nutrients were found in vermiwash but vermiwash II cold stress consisted better than others. Cold stress vermiwash performed well for the preparation of vermiwash which was found efficient in inducing seedlings growth.

Key words: *Eisienia foetida*, vermiwash, seed germination

Address for Correspondence:

S Senthilmurugan

Department of Zoology, Annamalai University,

Tamilnadu,608 002

Email Id: senthilmuruganphd@yahoo.co.in, India

INTRODUCTION:

As the world is becoming sensitive towards issues of environment, ecology and chemical in agriculture, the new era has come to think alternatives for chemical fertilizers, pesticides products and environment destruction parameters. Repercussions of indiscriminate use of agro chemicals in the form of pesticides, fungicides and fertilizers in agriculture are being felt in every walk of life, including threat to human beings, animals, flora, fauna and even to Mother Earth. Today soils are exhausted, overworked, depleted, and poisoned by synthetic chemicals. As a sequel to the realization, efforts are being made to explore the possibilities of searching for ecologically sound, economically viable and technologically competitive farming modules.

Conventional insecticides are among the most popular chemical control agents because they are readily available, rapid acting, and highy reliable. A single application may control several different pest species and usually forms a persistent residue that continues to kill insects for hours or even days after application. Because of their convenience and effectiveness, insecticides quickly became standard practice for pest control during the 1960's and 1970's. The pesticide poisoning cases and consequent death is the most important ecological disturbance due to high chemical pesticide residue in food chain. It is well known that chemical pesticides cause significant health hazards to man and other organism-Predators, particularly those at the top of a food chain, amass pesticides concentration many times greater than anywhere else on the environment. Organic farming produces good quality of food by using eco-friendly technology. It depends upon use of leguminous plants and microbial inoculation for nitrogen fixation, crop rotation, recycling of organic wastes and biological control methods. Utilization of earthworms and its natural activity minimize the problems to a great extent [1].

The multiple benefit of the vermiculture in various perspectives is waste management, waste land conservation, land reclamation, water filtration, soil fertility, animal protein and enhancement of plant production. Earthworms are more possible to effect quick change to agriculture by using vermicompost and vermiwash. Earthworms ooze out body fluid into the dorsal pores to survive in the environment. The body fluid collected from the earthworm without harming is called as vermiwash. The application of vermiwash is in seed germination, plant growth enhancement and peat management. Vermiwash has enzymes which are secretion of earthworms that stimulate the growth and yield of crops and even develop resistance in crop receiving this spray, such preparation would certainly have the soluble plant nutrients a part from some organic acids and mucus of earthworms and microbes[2].

Fenugreek is a legume, originally from southeastern Europe and western Asia, but grown now mainly in India and also in certain parts of Asia, northern Africa, Europe and the United States [3]. It is known as methi (Hindi) in India and also as Fenugrec (France), Bockshorklee (Germany), Koroha (Japan), Hulba (Arab), Pazhitnik (Russia) and Ku-Tou (China). The seeds find extensive use in Indian cuisine to flavor many foods including curry powders, spice blends and tea. Its leaves are also used as a green leafy vegetable in the diet. Fenugreek seeds are aromatic but bitter with carminative, galactogouge, antibacterial and antiviral properties. The seeds contain a central hard yellow embryo surrounded by a corneous and comparatively large layer of white, semi-transparent endosperm [4]. Rheological properties and emulsions of galactomannans of fenugreek seed endosperm are reported [5].

The application of vermiwash is in agriculture with respect to its origin, cost effectiveness, it availability, reliability and ecofriendlyness. The aim of the study was carried out to understand the influence of the vermiwash on the growth of *Trigonella foenum-graecum*

.

MATERIALS AND METHODS:

Earthworms

Earthworms *Eisenia fetida* taken were from Periyar Maniammai University, Vallam, Thanjavur, Tamilnadu, India The earthworms were identified morphologically using an identification key [6].

Seed collection

Fenugreek (*Trigonella foenum-graecum*) seeds used were procured from local market, Mysore, India through a well-established indigenous supplier.

Vermiwash preparations

Vermiwash was extracted by three different methods which are as follows:

Method I (heat stress method)

This method was adopted from method described by [7]. Well-grown adult earthworms Eisenia foetida were separated from casting materials by placing the worms in a plastic tub for some time. Then the earthworms were removed carefully from the casting materials and transferred to a glass beaker containing 500 ml of warm (40 °C temperature) distilled water and agitated for 5–6 min with a stirrer. Around 30 g of warms were taken. They were then removed immediately and added to another pre-sterilized

plastic container filled with water at room temperature. Here the worms were rinsed thoroughly to collect the remaining excretory and secretory products adhering to the body of the worms. The earthworms were then released back to the stock culture container. The light yellow straw-coloured contents of the glass beaker and plastic beaker were mixed and the solution was stored in sterilized dark colour glass bottle at 4 °C to be used for the experimental purpose.

Method II (cold stress application)

In this case, vermiwash was produced using method described by Pramanik (2010) with slight modification. Around 25 numbers having around 30 g of total weight of well-grown matured worms (E. foetida) of approximately equal length were applied cold stress by keeping them in chilled conditions, in ice cubes in beaker at -5 °C for 3–4 min. The earthworms were then transferred to a 500-ml glass beaker containing cold distilled (°C) water and kept for 7–8 min with occasional stirring. The worms were transferred to a sterilized beaker containing distilled water at room temperature and rinsed for around 2–3 min and then released to the stock culture container. The light yellow-coloured exudutes from the petridish, glass beaker and plastic beaker were all mixed together and the vermiwash was stored in dark-coloured sterilized glass bottle at 4 °C for experimental purpose.

The effect of vermiwash on growth rate of seedlings

Fifty seeds of fenugreek *Trigonella foenum-graecum* were soaked in each treatment. After germination, two days old ten seedling were assembled in every test tube containing each types of vermiwash which were diluted in different concentration (Vermiwash I-T1-0.50% and T2-1%; Vermiwash II T3-0.50% and T4-1%). The root of seedlings emerged into the treatments keeping at same level. Root length, root number development and shoot length were measured daily. After germination endosperm, seed coat and sprouts were separated manually and dried individually in an oven at 55–60 °C for 6 h to get the moisture content of 5.51%, 7.92% and 16.68% respectively. Dry weight was measured at the end.

RESULTS AND DISCUSSION:

The effect of the different concentration of vermiwash differed significantly ($p<0.05$) in root growth of seedling (Table 1). The pattern of root growth is illustrated in Figure 1. On the second day mean root length (3.45cm) in Ver II 1%, (2.59cm) were significantly superior than control. At 10 days ver II 1% produced higher (12.39cm) mean root length than all other treatments.

The effect of the different type of vermiwash was significantly ($p<0.05$) different in the development of number of roots (Table 2). The number of roots developed is showed in Figure 2. The maximum mean number of roots per seedling produced in ver I 1% was 21.8. Ver II 1% produced significantly superior roots to all other treatments. At 10 days the mean number of root development was followed 20.2 in ver II 1%, 17.1 in ver I 0.5%, 16.7 in ver I 0.5%, 15.7 in ver II 1% and 13.6 in control.

The effect of the different types of vermiwash were significantly ($p<0.05$) different in shoot growth of seedling at 6^{th} and 10^{th} days (Table 3). The pattern of shoot growth is shown in Figure 3. Vermiwash II 1% produced 9.72cm shoot height and was significantly superior to all other treatments at 10^{th} day.

Table 1: Comparisons of mean root growth in different types of vermiwash against control.

Vermiwash	Difference between means of root growth (cm)								
	2^{nd} day	3^{rd} day	4^{th} day	5^{th} day	6^{th} day	7^{th} day	8^{th} day	9^{th} day	10^{th} day
Ver I 0.5%	0.23*	0.43	0.54	0.55	0.61	0.61	0.84	0.91	1.04
Ver I 1%	0.32*	0.47	0.57	6.43	7.45	0.933	1.05	1.22	1.30
Ver II 0.5%	0.43*	0.58	0.65	0.84	1.24	1.54	2.03*	2.21*	2.30*
Ver II 1%	0.45*	.0.60*	0.79*	0.89*	1.03*	1.45*	154*	1.96*	2.68*

Comparisons significant at 0.05 level are indicated by * (Anova, Dunnet's test)

Ver I 0.5% –heat stress vermiwash Ver I 1%-heat stress vermiwash

Ver II 0.5% – cold stress vermiwash Ver II 1% –cold stress vermiwash

Table 2: Comparisons of mean number of root development in different types of vermiwash against control.

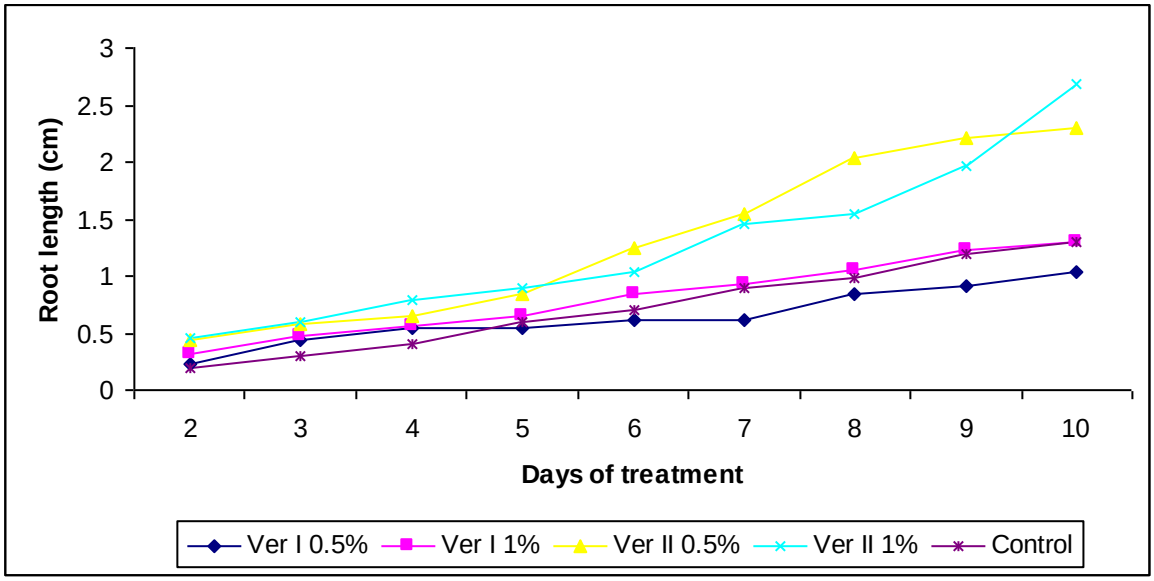
	Difference between means number of roots						
Vermiwash	4th day	5th day	6th day	7th day	8th day	9th day	10 th day
Ver I 0.5%	0.2	0.3	0.4	0.9	1.2	1.6	2.4
Ver I 1%	0.5	0.9	1.1	1.4	2	2.6	3.0
Ver II 0.5%	0.5	1.4	1.9	2.1	2.3	2.5	3.4
Ver II 1%	0.4*	0.9*	1.9	2.9*	3.4*	3.9*	4.2*

Comparisons significant at the 0.05 level are indicated by * (Anova, Dunnet's test)
Ver I 0.5% –heat stress vermiwash Ver I 1%-heat stress vermiwash
Ver II 0.5% – cold stress vermiwash Ver II 1% –cold stress vermiwash

Table 3: Comparisons of mean shoot growth in different types of vermiwash against control.

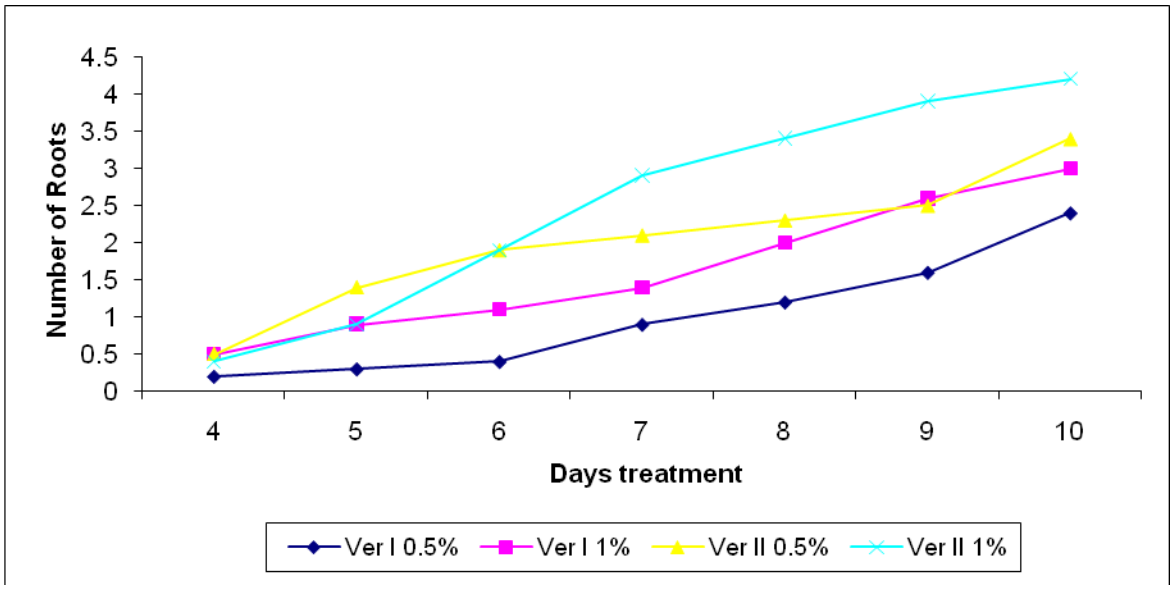
	Difference between means shoot growth						
Vermiwash	4th day	5th day	6th day	7th day	8th day	9th day	10 th day
Ver I 0.5%	0.2	0.5	0.7	0.9	1.5	1.9	3.2
Ver I 1%	0.4	0.9	1.1	1.4	2	2.0	2.5
Ver II 0.5%	0.4	1.3	1.6	2.0	2.1	2.4	2.8
Ver II 1%	0.5*	0.8*	1.4	2.0*	2.4*	2.9*	3.4*

Comparisons significant at the 0.05 level are indicated by * (Anova, Dunnet's test)
Ver I 0.5% –heat stress vermiwash Ver I 1%-heat stress vermiwash
Ver II 0.5% – cold stress vermiwash Ver II 1% –cold stress vermiwash



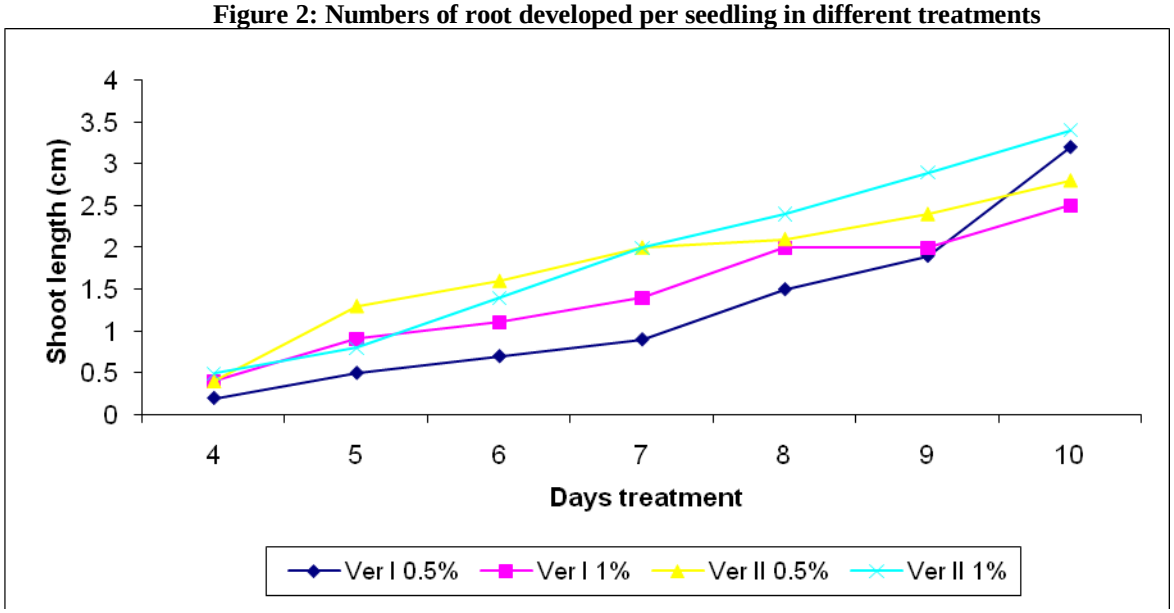
Ver I 0.5% –heat stress vermiwash Ver I 1%-heat stress vermiwash
Ver II 0.5% – cold stress vermiwash Ver II 1% –cold stress vermiwash

Figure 1: Root growth of seedling in different treatments



Ver I 0.5% –heat stress vermiwash Ver I 1%-heat stress vermiwash
Ver II 0.5% – cold stress vermiwash Ver II 1% –cold stress vermiwash

Figure 2: Numbers of root developed per seedling in different treatments



Ver I 0.5% –heat stress vermiwash Ver I 1%-heat stress vermiwash
Ver II 0.5% – cold stress vermiwash Ver II 1% –cold stress vermiwash

Figure 3*:* Shoot growth of seedling in different treatments

The reduction in total germination was higher for non-primed seeds, compared to primed seeds. Therefore, primed seeds maybe had better efficiency for water absorption and faster metabolic activities in seed during germination process [8]. According to [9] germination, growth, respiration, and other related processes can be affected in seeds that are subjected to salt stress changes in any one of these processes can affect other metabolic activities, particularly the carbohydrate metabolism that plays an important role in germination [10]. The reduction in final germination percentage can be explained by the increase of external osmotic pressure which affects the absorption of water by the seed and can be also due to the accumulation of Na+ and Cl- in the embryo which may lead to an alteration in the metabolic processes of germination and causes cells death in the embryo [11]. More specifically, this reduction in the germination rate, which was more pronounced in non-primed seeds, is related to the specific ionic salt stress during the second phase of germination that corresponds to the activation of embryo growth using reserve metabolites [12].

Fenugreek (Trigonella foenum-graecum L., family - Leguminoceae) an annual herb is well known as flavour, curry powder and spice [9,6]. It is originated in South Eastern Europe and West Asia. Today it is widely cultivated in India, Egypt, Argentina, China and America. Both seeds and green leaves are used as vegetables, food additives and fodder in South and Central Asian countries [13]. Fenugreek is grown for the purpose of green manuring in America. Fenugreek seeds are frequently used in human medicine to manage hypercholesterolemia, cancer and diabetes mellitus as they possess hypoglycemic, antilipidemic, anticarcinogenic and cholagogic properties [14]. Fenugreek is a short duration crop and is suitable for various cropping system. Soon after harvesting of main kharif crops like chillies, cotton and paddy, fenugreek is grown as rabi crop.

Vermiwash showed highest seedling growth compared to control. Ver II L was found superior in seedling growth to other types of vermiwash. Zambare *et al.* [8] reported that vermiwash showed high cowpea plant growth compared to without supplementation. Springett and Syers [15] observed that the earthworm altered nutrient availability, altered the plant ability to take up nutrient or affect growth mechanism of the plants. Therefore growth inducing substance and nutrition were found in all the vermiwash but ver II L performed better than others.

Fenugreek seeds are traditionally used for the treatment of many diseases. Studies show that the seeds have antioxidant properties. Many medicinal properties are attributed to fenugreek seeds and leaves [16]. Fenugreek is known to have several pharmacological attributes such as hypoglycaemic [17-19] and appetite stimulation [20].

The different types of vermiwash (Vermiwash I, Vermiwash II) influenced seedlings growth compared to control. Two different methods were used to collect the vermiwash from two species (0.5% and 1%). The root growth of seedlings was significantly higher in ver II 1% than other treatments. At 10 days both ver II 1% and ver II 1% produced 12.29cm and 11.38cm root length respectively and were significantly superior to other treatments[21-23]. Ver II L produced significant number of roots that is superior to all other treatments from start to end. At 10 days mean shoot height of seedlings was 15.56cm in ver I E and 15.57cm in ver II L. The maximum mean dry matter of seedlings was 31.32cm in ver II. Therefore ver II was superior in seedlings growth compared to other types of vermiwash. The growth inducing substance and nutrition found in the vermiwash influenced the growth rate and was higher than compared to control but ver II L consisted better than others. Local species (*E.fotida*) responded well could be used for the preparation of vermiwash which was efficient in seedlings growth.

REFERENCES:

1. Almansouri M, Kinet JM, Lutts S (2001). Effect of salt and osmotic stresses on germination in durum wheat (Triticum durum Desf.). Plant and Soil, 231: 243-254. Ashraf M (1994). Breeding for salinity tolerance in plants. Crit. Rev. Plant Sci. 13(1): 17-42.
2. Ashraf, M. and M.R. Foolad. (2005). Pre-sowing seed treatment – A shotgun approach toimprove germination, growth and crop yield under saline and non-saline conditions. Advances in Agronomy 88: 223-271.
3. Barasa, S.M.A., M. Farooq and R. Tabassum, (2005). Physiological and biochemical aspects of seed vigor enhancement treatments in fine rice (Oryza sativa L.). Seed Science and Technology, 33: 623-628.
4. Basra, S.M.A., I. Afzal, A. Hameed and R.A. Rashid. (2005). Inducing salt tolerance in wheat by seed vigor enhancement techniques. International Journal of Agriculture and Biology 2(1): 173-179.
5. Bose, B. and Mishra T. (1992). Response of wheat seed to presowing seed treatment with Mg(NO3)2. Ann. Agric. Res. 5: 11-16.
6. Botia P., Carvajal M., Cerda A., Martinez V. (1998). Response of eight Cucumis melo cultivars to salinity during germination and early vegetative growth. Agronomy, 18: 503-513.
7. Boursiac Y., Chen S., Luu D.T., Sorieul M., Dries N., and Maurel C. (2005). Early effects of salinity on water transport in Arabidopsis roots: molecular and cellular features of aquaporin

expression. Plant Physiol, 139: 790-805. Bradford, K.J. (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. Hort Science, 21: 1105-1112.
8. Cano, E.A., M.C. Bolarin, F. Perez-Alfocea and M. Caro. (1991). Effect of NaCl priming on increased salt tolerance in tomato. Journal of Horticultural Science 66: 621-628.
9. Cayuela E., Perez-Alfocea F., Caro M. and Bolarin M.C. (1996). Priming of seeds with NaCl induces physiological changes in tomato plants grown under salt stress. Physiology Plant., 96: 231–236.
10. Cicek, N. and H. Cakirlar. (2002). The effect of salinity on some physiological parameters in two maize cultivars. Bulgarian J. Plant Physiol. 28: 66-74. Demir, H. and K. Mavi, (2004). Seed priming of male sterile muskmelon (Cucumis melo L.) for low temperature germination. Seed Science and Technology, 23(4): 881-884.
11. Drihem, K. and D.J. Pilbeam. (2002). Effects of salinity on accumulation of mineral nutrients in wheat grown with nitrate-nitrogen or mixed ammonium:nitrate-nitrogen. J. Plant Nut., 25: 2091-2113.
12. Ehsanfar, S., S.A. Modarres-Sanavy and R.TavakkolAfshari, (2006). Effects of osmopriming on seed germination of canola (Brassica napus L.) under salinity stress. Communications in agricultural and applied biological sciences, 71(2): 155-159.
13. Eidi, A., Eidi, M. and Sokhteh, M. (2007). Effect of fenugreek (Trigonella foenum-graecum L.) seeds on serum parameters in normal and streptozotocin-induced diabetic rats. Nutrition Research, 27: 728-733.
14. Esmaielpour, B., K. Ghassemi-Golezani, F.R. Khoei, V. Gregoorian, and M. Toorchi, (2006). The effect of NaCl priming on cucumber seedling growth under salinity stress. Journal of Food, Agriculture and Environment, 4(2): 347-349.
15. Farhoudi R., Sharifzadeh F., Poustini K., Makkizadeh M.T., Kochakpor M. (2007). The effects of NaCl priming on salt tolerance in canola(Brassica napus) seedlings grown under saline conditions. Seed Sci. Tech., 35: 754-759.
16. Farooq, M., S.M.A. Basra, B.A. Saleem, M. Nafees and S.A. Chishti. (2005). Enhancement oftomato seed germination and seedling vigour by osmopriming. Pak. J. Agric. Sci., 42(3-4): 36- 41.
17. Foolad M.R., Lin G.Y. (1997). Genetic Potential for Salt Tolerance During Germination in Lycopersicon Species. Hort. Sci. 32: 296-300.
18. Gill, K.S. and O.S. Singh. (1985). Effect of salinity on carbohydrate metabolism during paddy (Oryzasativa L.) seed germination under salt stress condition. J. Exp. Biol. 23: 384-386.
19. Gill, P.K., Sharma, A.D., Singh, P. and Bhullar, S.S., (2003). Changes in germination, growth and soluble sugar contents of Sorghum bicolor (L.). Moench seeds under various abiotic stresses. Plant Growth Regulation, 40 (2), 157-162.
20. Hajlaoui H., Denden M., Bouslama M., (2007). Etude de la variabilité intraspécifique de tolérance au stress salin du pois chiche (Cicer arietinum L.) au stade germination. Tropicultura, 25: 168–173.
21. Hopper NW, Overholt JR, Martin JR (1979). Effect of cultivar, temperature and seed size on the germination and emergence of soy beans (Glycine max (L.) Merr.). Ann. Bot. 44: 301-308.
22. Kathiresan, K., V. Kalyani and J.L. Gnanarethium. (1984). Effect of seed treatments on field emergence, early growth and some physiological processes of sunflower (Helianthus annuus L.). Field Crops Res. 9: 255- 259.
23. Liu, Y., R.J. Bino, D. Van, W.J. Burg, S.P.C. Groot and H.W.M. Hilhorst. (1996). Effects of osmotic priming on dormancy and storability of tomato (Lycopersicon esculentum Mill.) seeds. Seed Science Research, 9: 49-55.