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International Journal of Current Research
Vol. 6, Issue, 11, pp.10387-10393, November, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EFFECT OF PRE SOWING HERBAL HARDENING TREATMENT ON INITIAL SEED QUALITY PARAMETERS IN SESAME (*Sesamum indicum L.*) cv. TMV 3

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ARTICLE INFO

Article History:
Received 15th August, 2014
Received in revised form
18th September, 2014
Accepted 24th October, 2014
Published online 30th November, 2014

Key words:

Sesame,
Herbal Extract,
Germination,
Electrical Conductivity

ABSTRACT

In vitro evaluation was carried out to study the effect of various herbal leaf extract seed hardening treatments on seed quality of sesame. The sesame seeds were soaked at 4, 6, 8, 10 and 12 hours of duration at 5, 10, 15 and 20 % concentrations of aqueous leaf extract of chicory, aswagantha, ocimum and sarpagandha plants. The study revealed that, the 20% concentration with 4 hours of all herbal extracts showed high increase in seed germination and vigour index. Best treatment from each herbal hardened seeds was confirmed in confirmative trials of laboratory with three different vigour lots of sesame. Significant activity was also observed in 20 % chicory leaf extract hardened with 4 hours of treatment, when compared to other treatments and control, which have recorded higher germination percentage in all the vigour lots (93 % in high vigour, 84 % in medium vigour and 73 % in low vigour seeds) and lower Electrical conductivity (0.075 dSm^{-1} in high vigour, 0.089 dSm^{-1} in medium vigour and 0.095 dSm^{-1} in low vigour seeds). But control recorded lower germination (81 % in high vigour, 67 % in medium vigour and 55 % in low vigour lots) and higher electrical conductivity (0.093 dSm^{-1} in high vigour, 0.124 dSm^{-1} in medium vigour, 0.146 dSm^{-1} in low vigour seeds).

INTRODUCTION

In India, oil seeds are the second largest commodity next to cereals. It shares 13 per cent of the country's gross cropped area. Sesame (*Sesamum indicum* L.) is one of the oldest oilseed plants used by man, and has great economic potential due to the possibilities for its exploitation in both the domestic and international markets. Seed being the basic input in agriculture, production and supply of quality seeds to the farmers will go a long way to achieve the goal of self sufficiency in oilseed crops. Out of many constraints regarding low production of oilseeds, seed quality is the prime importance. One of the most notable characteristics is that they loose their viability very easily (Denise de Castro Lima *et al.,* 2014). Seed treatment with chemicals are commonly used to ensure uniform stand establishment by improving germination and vigour and protecting against soil borne pathogens and insects. Many of synthetic fungicides are known for their non-biodegradable nature and residual toxicity (Pak, 2003). The ill effects associated with the use of chemical fungicides like carcinogenicity and teratogenicity may cause a serious health problem. Hence there is an urgent need to search for alternative strategies for effective seed management practice.

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Medicinal plants are natures' wonderful gift and used widely in traditional systems like Ayurveda, Siddha and Unani. Medicinal plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Recently much attention has been directed towards extracts and biologically active compounds isolated from popular plant species. In recent years, secondary plant metabolites (Phytochemicals) previously with unknown pharmacological activities have been extensively investigated as source of medical agents (Prince and Prabakaran, 2011).

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries. Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses. A wide range of medicinal plant parts is used to extract as raw drugs and they possess varied medicinal properties (Dash *et al.,* 2011). Hence the present study, aqueous leaf extract of various herbal plants i.e., chicory, aswagantha, ocimum and sarpagandha were evaluated for their potency in seed quality parameters in sesame.

MATERIALS AND METHODS

The present study was carried using genetically pure seeds of sesame (*Sesamum indicum L*) cv. TMV 3 obtained from the Oilseed Research Station, Thindivanam, Tamilnadu. The experiments were conducted at the Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar (11°24'N latitude and 79°44'E longitude with an altitude of +5.79 mts above mean sea level). The bulk seeds were graded for uniformity using appropriate round perforated metal sieves of sizes of 5/64".

Experiment 1. Standardization of Herbal Extract Hardening Treatment

Sesame seeds cv TMV 3 were soaked in 5, 10, 15 and 20 % concentrations of the aqueous leaf extract of herbal plants i.e., chicory, aswagantha, ocimum and sarpagandha for 4, 6,8, 10 and 12 hours duration.

Preparation of Herbal plant leaf extract

The fresh leaves of herbal plants i.e., chicory, aswagantha, ocimum and sarpagandha plants were collected separately and dried under shade. The shade dried leaves were powdered using mortar and pestle. Then 5, 10, 15 & 20 g of leaf powder were exactly weighed using weighing balance and dissolved in 100 ml of distilled water which was measured already in the beaker to make 5,10,15, and 20 % leaf extracts. The herbal leaf extracts was filtered by using muslin cloth to remove unwanted material and leaf debris. Then the seeds were soaked in the respective herbal leaf extract solutions at 1:1 (W/V) ratio of volume of seeds to herbal extract) along with water for different durations (4,6,8,10 and 12 hours) under aerated conditions at room temperature. Then the seeds were air dried under the shade for two days and in drying chamber maintained at 30 ± 0.5^{o} C for two days to bring back to their original moisture content. The treatments were evaluated for their seed quality parameters i.e., germination percentage (ISTA, 1999), shoot length (ISTA, 1999), root length (ISTA, 1999), dry matter production (ISTA, 1999) and vigour index (Abdul-Baki and Anderson, 1973).

Experiment 2. Confirmation Experiment

Based on the results from the first experiment, promising herbal hardening treatments were further evaluated in confirmative trials of laboratory with three vigour lots (High vigour - immediately harvest, Medium vigour- 3 months after harvest and low vigour- 6 months after harvest) of sesame seeds.

Treatment details

To - Control
T_1- Water Soaking
T_2 -Chicory (*Cichorium intybus*) Leaf extract hardening @ 20 % with 4 hour soaking
T_3- Aswagantha (*Withania somnifera*) Leaf extract hardening @ 20 % with 4 hour soaking.
T_4-Ocimum (*Ocimum gratissimum*) Leaf extract hardening @ 20 % with 4 hour soaking
T_5- Sarpagandha (*Rawolfia serpentina*) Leaf extract hardening @ 20 % with 4 hour soaking

The above treatments were evaluated for their seed quality parameters i.e., germination percentage (ISTA, 1999), speed of germination (Maguire, 1962), shoot length (ISTA, 1999), root length (ISTA, 1999), drymatter production (ISTA, 1999), electrical conductivity (Priestley and Leopold, 1983) and dehydrogenase enzyme activity (Kittock and Law, 1968) under laboratory condition. The data were statistically analyzed as per the method of Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Experiment 1. Standardization of Herbal Extract Treatment

Establishment of a good seedling stand in the field is an important and foremost need for higher crop yield. This depends largely on the field germination and vigour potential of the seeds used for sowing. The results presented in table 1 to 4 revealed that the pre sowing herbal hardening treatments improved the germination percentage, vigour index and seedling growth characteristics over untreated control irrespective of dose and duration of soaking. However, the best effect was realized from the higher dose with lesser duration of soaking, while the lower doses with higher duration of soaking had very marginal effect on seed quality parameters. In the present study, various concentrations of chicory herbal hardening treatments with different duration of soaking revealed that 20 % chicory leaf extract imposed with four hour soaking recorded the higher germination percentage (98 %), lengthier root (9.2 cm), lengthier shoot (9.9 cm), higher dry matter production (3.12 mg) and vigour index (1823), when compared to control and other treatments. The control treatment recorded only lower germination (80 %), root length (6.7 cm), shoot length (7.4 cm), dry matter production (2.72 mg) and vigour index 1088 (Table 1). The probable reason may be the presence of tannins and Saponins in the chicory leaf extract, which are the high molecular weight polyphenolic compounds and play a protective role in plants against micro-organisms, insects, unfavourable climatic conditions and damage by other organisms and prevent the seed coat damage and also their good phytochemical and antioxidant composition, which would play an important role in antioxidant defence system against endogenous free radicals (Rao and Sung, 1995).

Standardization experiments of various concentrations of aswagantha leaf extract hardening treatments with different duration of soaking revealed that 20 % aswagantha leaf extract imposed with four hour recorded the higher germination percentage (94 %), lengthier root (7.7 cm), lengthier shoot (10.0 cm), higher drymatter production (2.96 mg) and vigour index (1664) when compared to control and other treatments. The control treatment recorded only lower germination (80 %), root length (6.7 cm), shoot length (7.4 cm), dry matter production (2.72 mg) and vigour index 1088 (Table 2).

Table 1. Standardization of herbal seed treatment with different concentrations of Chicory leaf extracts and soaking duration in sesame

Treatments	Germination Percentage (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg 10 seedlings^{-1})	Vigour Index
To-Control	80 (63.25)	6.7	7.4	2.72	1088
Seed Treatment with Chicory leaf extract @ 5 % with					
T_1-4 Hour soaking	91 (72.11)	8.6	9.3	2.91	1583
T_2-6 Hour soaking	89 (71.26)	8.5	9.0	2.83	1513
T_3 -8 Hour soaking	86 (68.14)	8.3	8.7	2.81	1419
T_4-10 Hour soaking	85 (67.01)	8.1	8.4	2.77	1360
T_5-12 Hour soaking	80 (63.25)	7.8	9.1	2.73	1312
Seed Treatment with Chicory leaf extract @ 10 % with					
T_6-4 Hour soaking	96 (76.36)	9.0	9.6	3.03	1738
T_7-6 Hour soaking	93 (75.32)	8.7	9.1	2.98	1609
T_8 -8 Hour soaking	90 (71.12)	8.5	9.0	2.95	1548
T_9-10 Hour soaking	89 (71.26)	8.4	8.9	2.92	1495
T_{10}-12 Hour soaking	84 (67.25)	8.3	8.6	2.87	1378
Seed Treatment with Chicory leaf extract @ 15 % with					
T_{11}-4 Hour soaking	93 (75.26)	8.8	9.5	2.97	1655
T_{12}-6 Hour soaking	90 (71.12)	8.7	9.1	2.94	1557
T_{13} -8 Hour soaking	89 (70.26)	8.2	8.9	2.91	1477
T_{14}-10 Hour soaking	86 (68.62)	8.1	8.4	2.85	1393
T_{15}-12 Hour soaking	81 (64.31)	8.0	8.3	2.82	1280
Seed Treatment with Chicory leaf extract @ 20% with					
T_{16}-4 Hour soaking	98 (78.17)	9.2	9.9	3.12	1823
T_{17}-6 Hour soaking	96 (72.11)	9.0	9.7	3.09	1747
T_{18}-8 Hour soaking	94 (75.62)	8.9	9.5	3.02	1682
T_{19}-10 Hour soaking	93 (75.32)	8.7	9.3	2.97	1627
T_{20}-12 Hour soaking	88 (70.26)	8.5	9.0	2.91	1496
Mean	89 (70.74)	8.4	8.9	2.9	1513
CD (0.05 %)	1.89	1.4	0.029	0.04	288

Figures in parenthesis are Arcsine Transformed value

Table 2. Standardization of herbal seed treatment with different concentrations of Aswagantha leaf extracts and soaking duration in sesame

Treatments	Germination Percentage (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg 10 seedlings^{-1})	Vigour Index
To-Control	80 (63.25)	5.7	7.9	2.72	1088
Seed Treatment with Aswagantha leaf extract @ 5 % with					
T_1-4 Hour soaking	85 (67.01)	7.4	9.3	2.87	1419
T_2-6 Hour soaking	84 (67.21)	7.0	9.0	2.81	1344
T_3 -8 Hour soaking	83 (66.31)	6.7	8.9	2.77	1295
T_4-10 Hour soaking	81 (64.26)	6.2	8.6	2.70	1199
T_5-12 Hour soaking	77 (62.31)	6.0	8.1	2.69	1086
Seed Treatment with Aswagantha leaf extract @ 10 % with					
T_6-4 Hour soaking	88 (70.21)	7.4	9.5	2.89	1487
T_7-6 Hour soaking	87 (69.21)	7.1	9.3	2.84	1427
T_8 -8 Hour soaking	85 (67.01)	6.9	9.1	2.80	1360
T_9-10 Hour soaking	83 (66.62)	6.6	8.8	2.72	1278
T_{10}-12 Hour soaking	79 (63.00)	6.2	8.2	2.71	1138
Seed Treatment with Aswagantha leaf extract @ 15 % with					
T_{11}-4 Hour soaking	92 (73.32)	7.5	9.7	2.93	1582
T_{12}-6 Hour soaking	91 (72.26)	7.3	9.5	2.89	1528
T_{13} -8 Hour soaking	87 (69.25)	7.1	9.4	2.84	1435
T_{14}-10 Hour soaking	85 (67.06)	7.0	9.1	2.79	1368
T_{15}-12 Hour soaking	81 (64.26)	6.4	8.8	2.75	1231
Seed Treatment with Aswagantha leaf extract @ 20% with					
T_{16}-4 Hour soaking	94 (76.12)	7.7	10.0	2.96	1664
T_{17}-6 Hour soaking	92 (74.21)	7.5	9.9	2.91	1601
T_{18}-8 Hour soaking	90 (71.26)	7.3	9.7	2.86	1530
T_{19}-10 Hour soaking	89 (70.03)	7.1	9.2	2.81	1451
T_{20}-12 Hour soaking	82 (65.06)	6.6	8.9	2.79	1279
Mean	85 (67.06)	6.8	9.0	2.81	1370
CD (0.05 %)	1.2	0.13	0.11	0.01	212

Figures in parenthesis are Arcsine Transformed value

Table 3. Standardization of herbal seed treatment with different concentrations of Ocimum leaf extracts and soaking duration in sesame

Treatments	Germination Percentage (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg 10 seedlings^{-1})	Vigour Index
To-Control	80 (63.06)	5.7	7.9	2.72	1088
Seed Treatment with Ocimum leaf extract @ 5 % with					
T_1-4 Hour soaking	88 (70.03)	7.5	9.5	2.95	1496
T_2-6 Hour soaking	87 (69.25)	7.3	9.4	2.90	1453
T_3 -8 Hour soaking	85 (67.06)	7.0	9.0	2.88	1360
T_4-10 Hour soaking	84 (67.21)	6.7	8.7	2.80	1294
T_5-12 Hour soaking	81 (64.32)	6.5	8.3	2.72	1199
Seed Treatment with Ocimum leaf extract @ 10 % with					
T_6-4 Hour soaking	94 (76.32)	7.8	10.0	3.00	1673
T_7-6 Hour soaking	93 (74.52)	7.7	9.8	2.96	1627
T_8 -8 Hour soaking	90 (72.03)	7.4	9.5	2.90	1521
T_9-10 Hour soaking	87 (69.25)	7.1	9.1	2.88	1409
T_{10}-12 Hour soaking	84 (67.21)	6.9	8.7	2.82	1310
Seed Treatment with Ocimum leaf extract @ 15 % with					
T_{11}-4 Hour soaking	94 (76.12)	7.7	9.7	2.96	1636
T_{12}-6 Hour soaking	91 (72.11)	7.5	9.5	2.91	1547
T_{13} -8 Hour soaking	88 (70.33)	7.1	9.1	2.90	1426
T_{14}-10 Hour soaking	86 (68.21)	4.0	8.9	2.87	1109
T_{15}-12 Hour soaking	82 (65.26)	6.6	8.5	2.79	1239
Seed Treatment with Ocimum leaf extract @ 20% with					
T_{16}-4 Hour soaking	96 (76.23)	8.0	10.2	3.03	1747
T_{17}-6 Hour soaking	94 (76.14)	8.0	10.0	2.99	1692
T_{18}-8 Hour soaking	92 (74.48)	7.7	9.7	2.94	1601
T_{19}-10 Hour soaking	91 (72.17)	7.4	9.4	2.92	1529
T_{20}-12 Hour soaking	86 (68.67)	7.2	9.2	2.86	1410
Mean	88 (70.23)	7.0	9.2	2.88	1421
CD (0.05 %)	1.5	0.14	0.13	0.02	251

Figures in parenthesis are Arcsine Transformed value

Table 4. Standardization of herbal seed treatment with different concentrations of Sarpagandha leaf extracts and soaking duration in sesame

Treatments	Germination Percentage (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg 10 seedlings^{-1})	Vigour Index
To-Control	80 (63.35)	5.7	7.9	2.72	1088
Seed Treatment with Sarpagandha leaf extract @ 5 % with					
T_1-4 Hour soaking	86 (68.44)	7.2	9.4	2.89	1428
T_2-6 Hour soaking	85 (67.41)	7.1	9.1	2.86	1377
T_3 -8 Hour soaking	84 (67.21)	6.8	8.9	2.80	1319
T_4-10 Hour soaking	83 (66.31)	6.3	8.5	2.77	1262
T_5-12 Hour soaking	81 (64.67)	6.1	8.1	2.70	1150
Seed Treatment with Sarpagandha leaf extract @ 10 % with					
T_6-4 Hour soaking	94 (76.36)	7.6	9.7	2.93	1626
T_7-6 Hour soaking	92 (73.41)	7.4	9.5	2.90	1555
T_8 -8 Hour soaking	89 (70.63)	7.1	9.3	2.87	1460
T_9-10 Hour soaking	85 (67.09)	6.7	9.0	2.82	1334
T_{10}-12 Hour soaking	84 (67.64)	6.5	8.7	2.73	1277
Seed Treatment with Sarpagandha leaf extract @ 15 % with					
T_{11}-4 Hour soaking	91 (72.74)	7.5	9.5	2.90	1547
T_{12}-6 Hour soaking	89 (70.41)	7.3	9.3	2.87	1477
T_{13} -8 Hour soaking	86 (69.31)	7.0	9.0	2.85	1376
T_{14}-10 Hour soaking	84 (66.44)	6.5	8.8	2.80	1285
T_{15}-12 Hour soaking	83 (65.98)	6.2	8.5	2.70	1220
Seed Treatment with Sarpagandha leaf extract @ 20% with					
T_{16}-4 Hour soaking	95 (73.64)	7.9	10.1	2.97	1710
T_{17}-6 Hour soaking	93 (74.56)	7.7	9.8	2.95	1627
T_{18}-8 Hour soaking	90 (72.31)	7.3	9.6	2.91	1521
T_{19}-10 Hour soaking	88 70.39)	7.0	9.3	2.87	1434
T_{20}-12 Hour soaking	87 (69.38)	6.8	9.1	2.81	1383
Mean	87 (69.27)	6.9	9.1	2.83	1402
CD (0.05 %)	1.5	0.21	0.27	0.03	278

Figures in parenthesis are Arcsine Transformed value

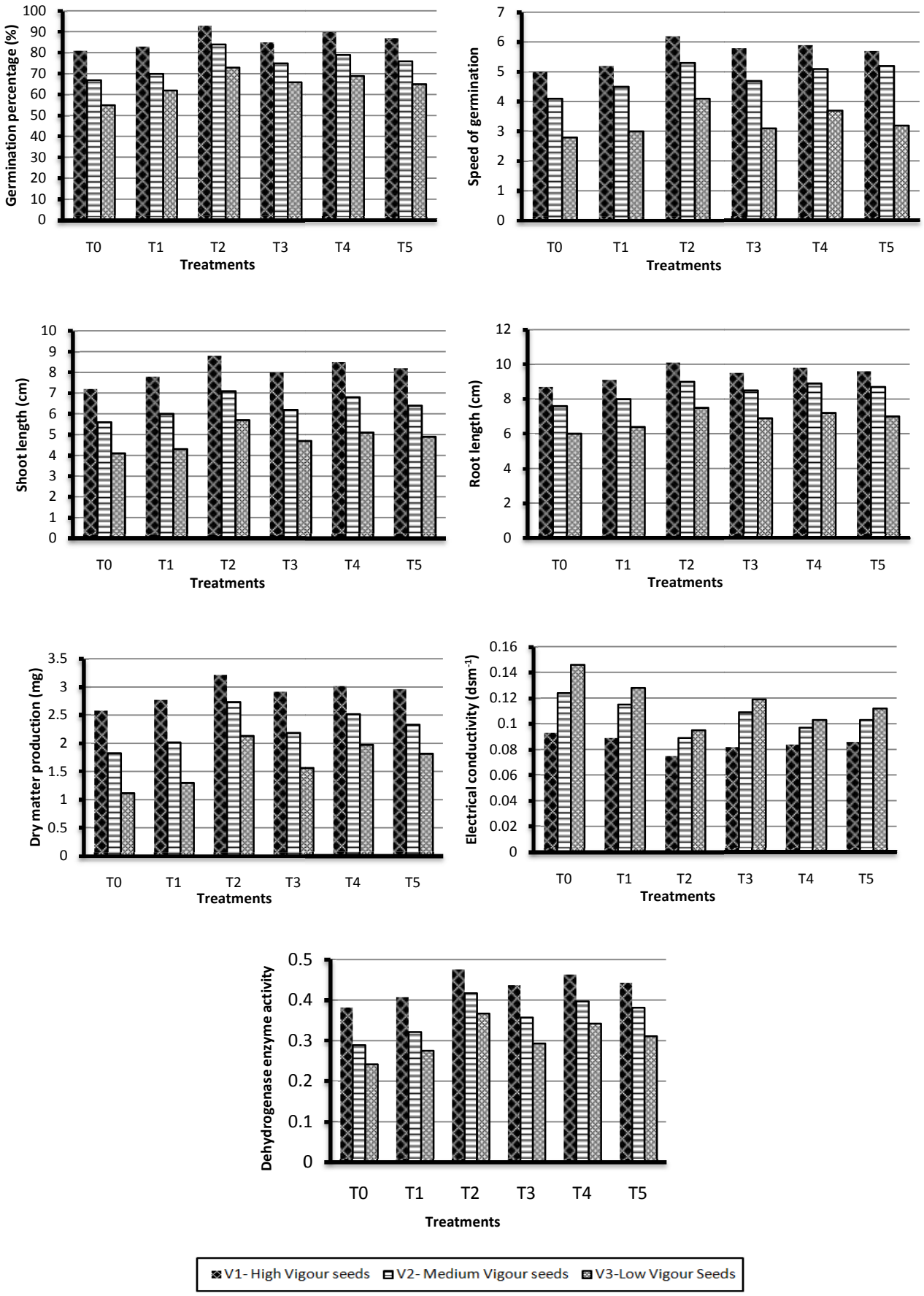

Figure 1. Effect of different medicinal plant leaf extracts on seed quality in sesame cv TMV 1 seeds.

This was due to the stimulatory effect of withanine and somniferne alkaloids which are present in the aswagantha leaf extract which are considered to improve growth behaviour and development of biosynthesis of secondary metabolites in various plants (Steward and Krikorian, 1971). Standardization of various concentrations of ocimum leaf extract hardening treatments with different duration of soaking revealed that 20 % ocimum leaf extract imposed with four hour recorded the higher germination percentage (96 %), lengthier root (8.0 cm), lengthier shoot (10.2 cm), higher drymatter production (3.03 mg) and vigour index (1747) when compared to control and other treatments. The control treatment recorded lower germination (80 %), root length (6.7 cm), shoot length (7.4 cm), dry matter production (2.72 mg) and vigour index 1088 (Table 3). It might be due to the effect of biologically active phytochemicals i.e., eugenol, methyl eugenol and caryophyllene which are present in ocimum leaf extract which will induce cytokinin and other plant growth stimulent secretion in enhancing the proliferation of shoot and root of developing plants (Vanitha *et al.*, 2008).

Standardization of various concentrations of sarpagantha leaf extract hardening treatments with different duration of soaking revealed that 20 % sarpagantha leaf extract imposed with four hour recorded the higher germination percentage (95 %), lengthier root (7.9 cm), lengthier shoot (10.1 cm), higher drymatter production (2.97 mg) and vigour index (1710) (Table 4) when compared to control and other treatments. The control treatment recorded only germination (80 %), root length (6.7 cm), shoot length (7.4 cm), dry matter production (2.72 mg) and vigour index 1088 (table 4). It may be due to the stimulatory effect of resperine, principle alkaloid from sarpagandha leaf extract (Reeta Kumari *et al.,* 2013) which may stimulate early germination by stimulating the synthesis of hydrolysing enzymes which degrade the food reserves for growing seedling which in turn leading to enormous growth (Brady and McCourt, 2003).The results are in conformity with the findings of Sindhu *et al*. (2013) in green gram and Sujatha (2006) in cowpea. Based on the above study, it was concluded that sesame seeds hardened with various medicinal herbal leaf extracts i.e, chicory, aswagantha, ocimum and sarpagandha plants @ 20 % concentration with four hour soaking recorded higher values and performed better for improving seed qualities in sesame.

Experiment 2. Confirmation Experiment

Conformation laboratory experiment revealed that among the various herbal hardening treatments, 20 % chicory leaf extract hardening with 4 hour soaking treatment significantly improved the various seed qualities observed. The above treatment recorded higher germination percentage, speed of germination, root length, shoot length, drymatter production and dehydrogenase enzyme activity and lower electrical conductivity when compared with other treatments and control in all vigour seeds of sesame (Fig. 1). This treatment also recorded higher germination percentage i.e., 93 % in high vigour seeds, 84 % in medium vigour seeds and 73 % low vigour seeds and lower electrical conductivity i.e., (0.075 dSm^{-1} in high vigour seeds, 0.089 dSm^{-1} in medium vigour seeds and 0.095 dSm^{-1} in low vigour seeds). The control seeds recorded lower germination percentage i.e., 81 % in high vigour seeds, 67 % in medium vigour seeds and 55 % in low vigour seeds and higher electrical conductivity i.e., 0.093 dSm^{-1} in high vigour seeds , 0.124 dSm^{-1} in medium vigour seeds and 0.146 dSm^{-1} in low vigour seeds. The probable reason might be the leaf extract of chicory having various phyto chemical components i.e., tannins, saponins, flavonoids, terpenoids, which are non nutritive compounds and play an important role in various functions of growth, thereby improving oxidative process of important marcromolecules such as lipids, proteins and nucleic acids It would have triggerred the germination process earlier thereby utilizing the available nutrients. This invigourative effect might have rectified the causes of low vigour and improved the seed and seedling characteristics (Shad *et al.,* 2013). They are participating in the antioxidant defence system against endogenous free radicals. These compounds possess hydrogen donating abilities and thus exert their antioxidant effect by breaking the free radical chain and prevent the cell membrane (Lobo *et al*., 2010). The above results are in conformity with the reports of Vanitha *et al.* (2008) in maize and Vanitha *et al.* (2009) in sunflower. Thus, the effect of pre sowing herbal hardening treatment on seed quality in sesame revealed that 20 % chicory leaf extract hardened with four hour soaking recorded higher initial seed quality when compared to other treatments and control.

Acknowledgment

The Authors are very much thankful to UGC, Government of India, New Delhi for the Financial support in the form of major research project.

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