

Effect of Medicinal Plants Leaf Extract on Seed-borne mycoflora, Seed Germination and Seedling Health of Spinach

¹M. A. Patekar, ²R. M. Kadam

¹Assistant Professor, ²Assistant Professor and Head,

¹PG Department of Botany, ²Department of Botany

¹Shivaji Mahavidyalaya, Udgir, Dist: Latur, ²Mahatma Gandhi Mahavidyalaya, Ahmedpur, Dist: Latur (MS), India

Abstract: Spinach seed samples collected from *Marathwada* region has been used for the detection of seedborne mycoflora. Nine seed-borne fungi have been identified viz. *Aspergillus flavus*., *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria alternata*, *Fusarium oxysporum*, *Rhizopus stolonifera*, *Penicillium digitatum*, *Cladosporium* spp. and *Curvularia* spp. Ten different plant extracts were tested for seed treatment at 10.0 % concentration. Treatments with leaf extracts of all plants showed significantly increased percentage seed germination over control (15.15 % to 45.45 %). The germination failure significantly decreased with increased vigour index and seedling health.

Key words – Leaf extracts, seed-borne mycoflora, seedling health

I. INTRODUCTION

Vegetables are important part of healthy eating and provides source of many nutrients including potassium, fibers, foliates and vitamins A, B and C. Vegetables are important source of farm income because they give more yield per unit time than cereals and other crops. Spinach is one of the most common leafy vegetable of tropical and sub-tropical regions of India. The popular spinach cultivating states include Uttar Pradesh, West Bengal, Maharashtra and Gujarat. It is a rich source of Vitamin A and C and also contains appreciable amount of protein, calcium, iron and anti-oxidants. Spinach is most probably a native of Indo-Chinese region. During cultivation, farmers face a big problem of susceptibility of vegetables towards various diseases during growing phase and most of them are seed-borne. Seed-borne *Cladosporium* spp. Causes Leaf spot (Hernandez-Perez and Toit., 2006), *Fusarium oxysporium* f. spp. *spinaciae* causes Wilt of spinach (Gatch and Toit, 2017). Thus, during cultivation, seed treatment with suitable seed protectant is an important aspect in integrated disease management. Seed protection is the application of a treatment to protect seeds from seed-borne and soil-borne disease organisms such as *Fusarium*, *Phythium* and *Rhizoctonia* which can cause pre-emergence damping-off, seed rot and seedling blight of many crops. (Mancini and Romanazzi, 2013).

Today medicinal plant extract has been accorded a lot of importance for crop protection against pest and diseases due to their target specificity and safety. Botanicals like leaf, root, stem, rhizomes, bulbs and other plant parts are used as extracts to control seed-borne pathogens by seed treatment. (Sangvikar and Wadje, 2012). During this investigation, ten medicinal plant leaf extracts were used for seed treatment and the results were found very promising with all treatments responsible to reduce seed mycoflora, seed germination failure and with enhanced seed germination, vigour index and seedling health. Kadam *et al.* (2008) also studied the efficacy of leaf extract of *Azadirachta indica* against seed borne fungi of groundnut. Their results indicated that the longer duration of seed treatment with plant extract was effective in controlling the growth of all surface borne seed mycoflora. Telang (2010) worked on similar lines with Chilli seed mycoflora and found that the seed treated with leaf extracts of *Azadirachta indica*, leaf and root extracts of *Ocimum sanctum* and leaf extracts of *Murraya koenigii* showed reduced incidence of seed mycoflora and maximum seed germination.

II. RESEARCH METHODOLOGY

The experiment was carried out at Aerobiology research center of Mahatma Gandhi Mahavidyalaya, Ahmedpur, Dist: Latur, Maharashtra. Two randomly selected seed samples (250 gm each) were collected using method described by Neergaard (1973). The seeds were collected from local farmers and market places of *Marathwada* region of Maharashtra state of India.

Spinach (*Spinacia oleracea*)

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2.1 Treatment of medicinal plant extracts on vegetable seeds:

Ten common plants namely *Azadirachta indica* A. Juss, *Aegle marmelos* Corr., *Calotropis procera* L., *Catharanthus roseus* L., *Datura metel* L., *Moringa oleifera* Lam., *Murraya koenigii* L., *Polyalthia longifolia* Sonn., *Ocimum sanctum* L. and *Tridax procumbens* L. were selected. The identification of plants was confirmed using the flora of Marathwada (Naik, 1998). These plants were surface sterilized with 0.1% HgCl₂ and washed repeatedly with sterile distilled water for three times. Vegetable seeds were treated by soaking the seeds in 10.0% concentration for 30 minutes with each of the plant extracts. Treated seeds were dried on blotter paper sheet in sun light for 30 minutes. The treated seeds were grown and incidence of fungi, rate of germination, vigour index and seedling health were studied by using blotter paper method as described by International Seed Testing Association (ISTA, 1966)

Table 1: Plants used for seed treatment

Sr. No.	Local Name	English Name	Scientific Name	Family
1	Neem	Indian lilac	<i>Azadirachta indica</i> A. Juss	Meliaceae
2	Bel	Indian quince	<i>Aegle marmelos</i> Corr.	Rutaceae
3	Ruchki	Rubber bush	<i>Calotropis procera</i> L.	Asclepiadaceae
4	Sadafuli	Periwinkle	<i>Catharanthus roseus</i> L.	Apocynaceae
5	Dhotara	Thorn apple	<i>Datura metel</i> L.	Solanaceae
6	Shevga	Drumstick	<i>Moringa oleifera</i> Lam.	Moringaceae
7	Kadhipatta	Curry leaves	<i>Murraya koenigii</i> L.	Rutaceae
8	Ashok	Mast tree	<i>Polyalthia longifolia</i> Sonn.	Annonaceae
9	Tulsi	Holy basil	<i>Ocimum sanctum</i> L.	Lamiaceae
10	Tantani	Coat buttons	<i>Tridax procumbens</i> L.	Asteraceae

Preparation of aqueous extracts:

Green leaf samples (100gm) were collected and washed very carefully with distilled water. Then plant parts were ground with conventional grinder called '*Mortar and pestel*' which is available and popular in every Indian farmer's house. Then grounded material were dipped in to 100 ml distilled water for 48 hours for complete extraction of the active ingredient from the extracted samples (Ahmed *et al.*, 2013). After that the water and ground material were filtered with the help of muslin cloth. This extract filtered with the help of Whatman's grade filter paper no. 1. Then crude extracts were preserved in glass bottles and kept in refrigerator at 4 +/- 2⁰C for further use.

2.2 Formulas used

For observations and results, following formulas were used:

$$\text{Percentage incidence of fungus} = \frac{\text{No. of seeds containing particular fungus}}{\text{Total Seeds}} \times 100$$

$$\text{Percentage incidence of seed mycoflora} = \frac{\text{No. of seed infected by fungi}}{\text{Total no. of Seeds}} \times 100$$

$$\text{Percentage germination} = \frac{\text{No. of seeds germinated}}{\text{Total no. of Seeds}} \times 100$$

$$\text{Vigour Index} = \text{Percentage germination} \times \text{Length of seedling}$$

2.2 Data Analysis

The experiment has been conducted in completely randomised design with three replications. All the observation data were compiled, tabulated and put to statistical computation for the presentation and interpretation of the results. Analysis of variance (ANOVA) was prepared for each study. Standard error between the means (SEm), critical difference (C.D.) and coefficient of variation (C.V.).

III. EXPERIMENTAL RESULTS

3.1 Effect of plant extracts on seedborne mycoflora, seed germination and vigour index:

Result depicted in Table 2, Fig. 1 and Plate I revealed that all plant extracts tested for seed treatment of Spinach were found to be most effective against seedborne mycoflora and gave significant increased seed germination, seedling length and vigour index compared to control. There was wide variation in fungal incidence ranging from 3.3 % under the influence of *Murraya koenigii* L. to 63.3 % in Control. Untreated seeds of spinach recorded 63.3% incidence of seedborne mycoflora, 66.7% seed germination with 293.48 vigour index. All treated seeds recorded lower incidence of seedborne mycoflora (3.3% to 23.3%) compared to untreated seeds. Among the tested plant extracts; *Azadirachta indica* A. Juss (3.3%) recorded lowest incidence of seedborne mycoflora followed by *Murraya koenigii* L. (3.3%), *Ocimum sanctum* L. (6.7%), *Moringa oleifera* Lam (10.0%) and *Calotropis procera* L. (13.3%). Moreover, plant extract significantly controlled the incidence of seed mycoflora with improved growth characteristic including germination percentage, seedling length and vigour index. The percentage seed germination was ranged from 66.7 % in control to 96.7 % under the influence of *Azadirachta indica* A. Juss. and *Murraya koenigii* L. Highest percentage germination was recorded by seeds treated with extract of *Azadirachta indica* A. Juss and *Murraya koenigii* L. (96.7% each), followed by *Aegle marmelos* Corr., *Calotropis procera* L., *Moringa oleifera* Lam. and *Ocimum sanctum* L. (93.3% each). Whereas; *Catharanthus roseus* L., *Datura metel* L. and *Tridax procumbens* L. seeds recorded 90%, 86.7% and 80% seed germination respectively. The seedling length of spinach ranged from 4.4 to 5.4 cm. The highest seedling length was recorded in *Azadirachta indica* A. Juss extract treated seeds (5.4cm.) followed by *Catharanthus roseus* L. and *Ocimum sanctum* L. (5.3 and 5.2 cm. respectively). Seed treatment with different plant extract were also improved the vigour index of spinach seeds over the control seeds. Highest vigour index (522.18) was recorded in spinach seeds treated with *Azadirachta indica* A. Juss leaf extract followed by *Aegle marmelos* Corr. and *Ocimum sanctum* L. gave statically identical results (485.16) for vigour index. *Calotropis procera* L. and *Murraya koenigii* Lam. leaf extract treated seeds also gave statically similar results (466.5 and 464.16 respectively) for vigour index of spinach. All rest of plant extract showed significant results in increasing the vigour index over the control.

Overall, treatment with leaf extracts of all plants significantly increased percent seed germination over the control. The seedling length was 4.4 cm. in control. It increased within the range of 4.8 cm. to 5.4 cm., the increase in seedling length was statistically significant at $p = 0.01$. The vigour index also significantly increased under the influence of leaf extracts from 293.48 in control to within the range of 368.16 to 522.18 under the influence of leaf extracts of various plants. Among all of the leaf extracts tested, the leaf extracts of *Azadirachta indica* A. Juss and *Ocimum sanctum* L. were found to be most effective.

Table 2: Effect of plant extracts on seed borne mycoflora, seed germination and vigour index of Spinach.

Sr. No.	Leaf Extract	Incidence of mycoflora (%)	Seed germination (%)	Seedling length (cm.)	Vigour index
1	Control	63.3	66.7	4.4	293.48
2	<i>Azadirachta indica</i> A. Juss.	3.3	96.7	5.4	522.18
3	<i>Aegle marmelos</i> Corr.	16.7	93.3	5.2	485.16
4	<i>Calotropis procera</i> L.	13.3	93.3	5	466.5
5	<i>Catharanthus roseus</i> L.	20	90	5.3	477
6	<i>Datura metel</i> L.	16.7	86.7	5.1	442.17
7	<i>Moringa oleifera</i> Lam.	10	93.3	4.9	457.17
8	<i>Murraya koenigii</i> L.	3.3	96.7	4.8	464.16
9	<i>Polyalthia longifolia</i> Sonn.	23.3	76.7	4.8	368.16
10	<i>Ocimum sanctum</i> L.	6.7	93.3	5.2	485.16
11	<i>Tridax procumbens</i> L.	23.3	80	5.1	408
	Mean	20.5909	87.8818	5.0182	442.6491
	SD	14.1234	9.1187	0.2691	61.3209
	CV	68.5904	10.3761	5.3618	13.8532
	SE	4.2584	2.7494	0.0811	18.4889
	CD 5%	9.4961	6.1311	0.1809	41.2304
	CD 1%	13.4990	8.7156	0.2572	58.6099

3.2 Effect of plant extracts on seed germination, germination failure and seedling health of spinach:

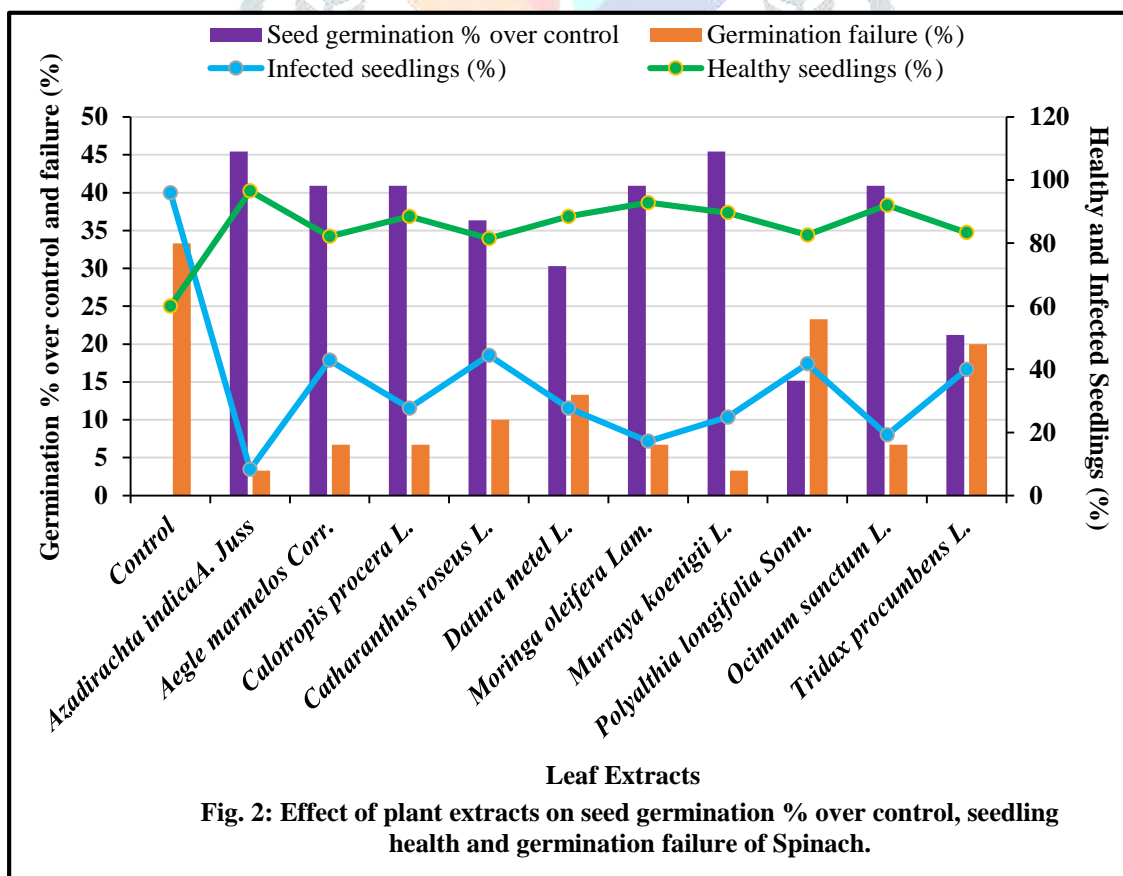
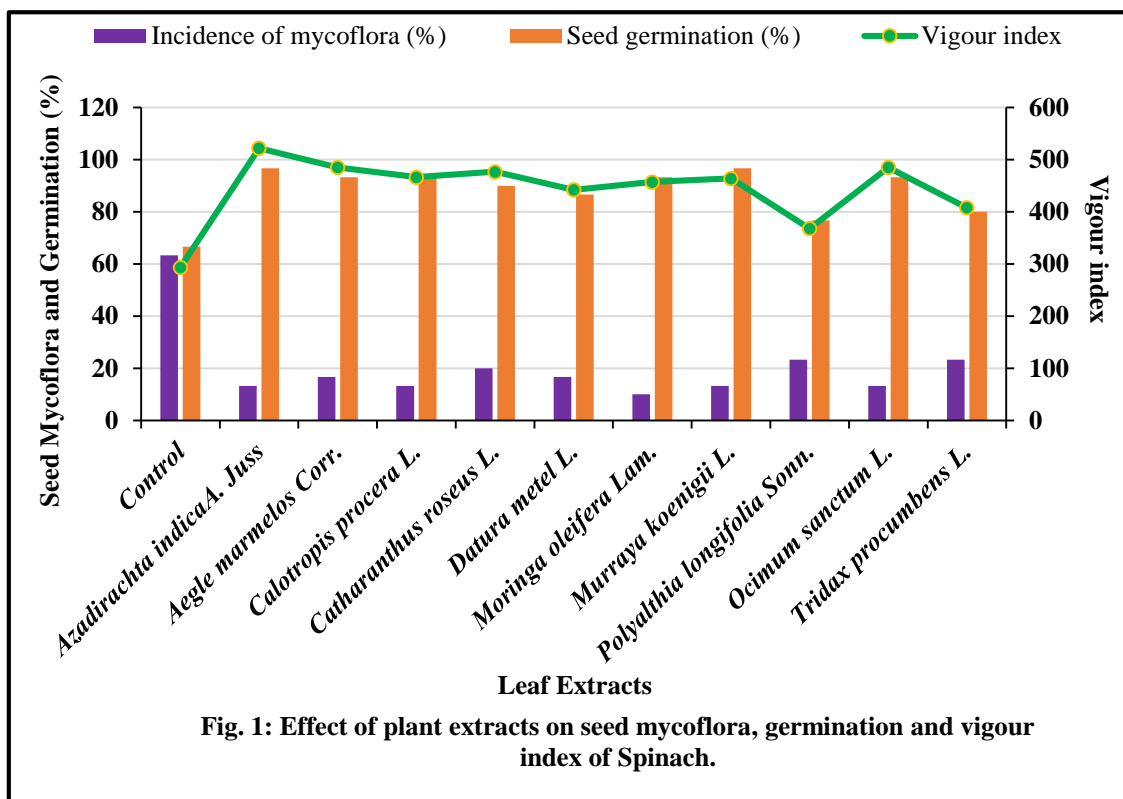
Aqueous extract of all tested plants on seed germination and seedling health of spinach showed ability to enhance seed germination and seedling health with reducing germination failure and infected seedling compared to control as summarized in Table 3 and Fig. 2. Highest (96.7%) seed germination was recorded with application of *Azadirachta indica* A. Juss. and *Murraya koenigii* L. extracts; which was 45.45% higher over untreated seeds. While the application of *Aegle marmelos* Corr., *Calotropis procera* L., *Moringa oleifera* Lam. and *Ocimum sanctum* L. leaf extract on seed recorded 40.9% (each) increased seed germination over control followed by *Catharanthus roseus* L. (36.36%). Treated seeds by other plant extracts also showed good result in respect and increased seed germination and decreased seed germination failure. Lowest germination failure (3.3%) was observed when seeds were treated with *Azadirachta indica* A. Juss. and *Murraya koenigii* L. extracts followed by *Aegle marmelos* Corr., *Calotropis procera* L., *Moringa oleifera* Lam. and *Ocimum sanctum* L. (6.7% each) leaf extracts.

The highest (96.55%) healthy and lowest (3.45%) infected seedling were obtained when the seeds treated with *Azadirachta indica* A. Juss. followed by *Moringa oleifera* Lam. with 92.85% healthy seedling and 7.15% infected seedling. But in case of untreated seeds 60.0% healthy and 40.0% infected seedlings were recorded. Treated seeds from *Ocimum sanctum* L. extract also showed good effect with healthy seedling of 92.0% and least infected seedling with 8.0%.

The percentage seed germination increased due to the treatment of leaf extracts. The percentage increase in seed germination ranged from 15.15 to 45.45%. The germination failure significantly decreased due to the treatment with leaf extracts.

Table 3: Effect of plant extracts on seed germination, seedling health and germination failure of Spinach.

Sr. No.	Leaf Extract	Germination (%)	Germination % over control (+)	Germination failure (%)	Healthy seedlings (%)	Infected seedlings (%)
1	Control	66.7	0	33.3	60	40
2	<i>Azadirachta indica</i> A. Juss	96.7	45.45	3.3	96.55	3.45
3	<i>Aegle marmelos</i> Corr.	93.3	40.9	6.7	82.14	17.86
4	<i>Calotropis procera</i> L.	93.3	40.9	6.7	88.46	11.54
5	<i>Catharanthus roseus</i> L.	90	36.36	10	81.48	18.52
6	<i>Datura metel</i> L.	86.7	30.3	13.3	88.46	11.54
7	<i>Moringa oleifera</i> Lam.	93.3	40.9	6.7	92.85	7.15
8	<i>Murraya koenigii</i> L.	96.7	45.45	3.3	89.65	10.35
9	<i>Polyalthia longifolia</i> Sonn.	76.7	15.15	23.3	82.6	17.4
10	<i>Ocimum sanctum</i> L.	93.3	40.9	6.7	92	8
11	<i>Tridax procumbens</i> L.	80	21.21	20	83.34	16.66
	Mean	87.8818	32.5018	12.1182	85.23	14.77
	SD	9.1187	13.8896	9.1187	9.2594	9.2594
	CV	10.3761	42.7347	75.2480	10.8640	62.6904
	SE	2.7494	4.1879	2.7494	2.7918	18.9019
	CD 5%	6.1311	9.3389	6.1311	6.2257	42.1511
	CD 1%	8.7156	13.2755	8.7156	8.8500	59.9189



IV. DISCUSSION

In case of Spinach seeds, nine different fungi viz. *Aspergillus flavus*., *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria alternata*, *Fusarium oxysporum*, *Rhizopus stolonifera*, *Penicillium digitatum*, *Cladosporium* spp. and *Curvularia* spp. were found to be associated. A considerable number of seed borne fungal pathogens belonging to the genera *Aspergillus*, *Alternaria*., *Curvularia* spp., *Fusarium* spp. and *Penicillium* spp. had been detected in spinach seed by many researchers (Baig *et al.*, 2012 and Islam *et al.*, 2012).

Vegetables and their seeds are vulnerable to many diseases including different pathogens. Fungal diseases of vegetables are caused by species of *Aspergillus*, *Fusarium*, *Alternaria*, *Rhizoctonia* and other pathogens. (Telang, 2010; Islam and Faruq, 2012; Survase, 2012; Sadda and Verma, 2015; Sultana and Gaffar, 2013; Nwokocha *et al.*, 2016). Seeds severely infected with fungi have poor seed quality such as germination and vigour. Many workers worldwide made effort to identify effective chemical fungicide to control fungal disease and also to improve seed germination and nutritional qualities but no chemical fungicide can control fungal disease completely and improve the seed quality. (Akinbode and Ikotum, 2008; Zacharia and Philip, 2010; Fayadh and Aledani, 2011). In addition, continuous application of chemical fungicides has created many environmental hazardous and residual problems. So, the only alternative for this problem is biological control. By considering the importance of alternative control method, the present work has been undertaken.

In medicinal plants, secondary metabolites are present. Phytochemical analysis of medicinal plants worked by Chiejina and Ukeh (2012) reported the presence of Tannins, Phlobatannins, Steroids, Terpenes, Saponins, Flavonoids and Alkaloids. The presence of these phenolic compound in the extracts indicates that these plants can serve as antimicrobial agents. In the present study, all treated seeds recorded lower incidence of seed borne mycoflora (3.3 % to 23.3 %) compared to untreated seeds (63.3 %). Vigour index also significantly increased under the influence of leaf extracts from 293.48 in control to within the range of 368.16 to 522.18. The percentage increase in seed germination ranged from 15.15 % to 45.45 %. The germination failure significantly decreased with enhanced seedling health. Survase (2012) evaluated the effect of medicinal plants leaf extract of ten plants on the seed mycoflora, seedling emergence and growth of seed borne fungi of methi. He reported that leaf extract of all the test medicinal plants were found to be inhibitory in more or less degree for incident of seed mycoflora. All the selected plants plant except *Vitex negundo* were found to be stimulatory for the seed germination and seedling emergence of methi. The leaf extract of *Semecarpus ancardium* (88.0%), *Solanum xanthocarpum* (79.0%), *Abrus precatorius* (81.0%), *Aegle marmelos* (84.0%), were found to be more inhibitory for incidence of seed mycoflora and more stimulatory for the seed germination and seedling emergence of methi.

Baka (2014) tested aqueous extracts from five wild traditional medicinal plants (*Achillea fragrantissima*, *Balanites aegyptiaca*, *Peganum harmala*, *Rumex vesicarius*, and *Urtica urens*) against the predominant fungal pathogens (*Alternaria alternata* f. sp. *lycopersici*, *A. solani*, *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani*) on infested Tomato seeds. He mentioned that, all the aqueous plant extracts significantly inhibited the mycelial growth and spore germination of these fungi as well as increased seed germination, plant emergence and seedling vigor was recorded. It is expected to be a natural phenomenon that the percent of seed germination and seedling health would be increased in case of treated seeds than that of untreated seeds. Similarly, many workers found the same phenomenon with different treatments. (Van Der Wolf *et al.*, 2008; Gupta *et al.*, 2012; Ahmed *et al.*, 2013; Bhale and Chatage, 2015; Islam *et al.*, 2011; Marraiki, 2013; Baka, 2014; Gayathri and Madhuri 2014; Hubert *et al.*, 2015; Sharma *et al.*, 2015; Signaboubo *et al.*, 2015)

V. CONCLUSION

All the tested medicinal plants have been found to be very effective against seedborne pathogens and showed increased seed germination, vigour index and seedling health. Thus, biological control methods should be preferred as one of the better alternative as they have minimum environmental impact in contrast to synthetic pesticides.

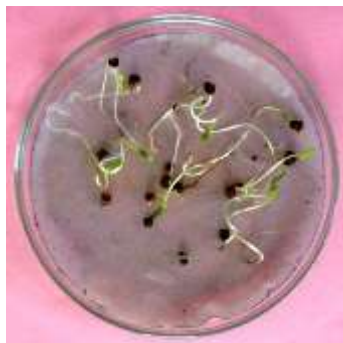
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Control



Azadirachta indica A. Juss



Aegle marmelos Corr.



Calotropis procera L.



Catharanthus roseus L.



Datura metel L.



Moringa oleifera Lam.



Murraya koenigii L.



Polyalthia longifolia Sonn.



Ocimum sanctum L.



Tridax procumbens L.

Plate I - Efficacy of plant extracts on seeds of Spinach