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Phytotoxic Potiential of Fertility Enhancing Medicinal Plants Prescribed in Sindh Pakistan

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Abstract: Pakistan has long history to use medicinal plants as an alternative medicine. Excessive intake of plant based bioactive compounds some time lead to cellular damages. In this regard evaluation of non-cytotoxic dose to human can initially be tested in plant based assay. Therefore, current work mainly focuses phytotoxic effects of different of radish seeds (R.S.) Rhizome of Land-calotrops root (L.R.) that were partitioned in germination inhibition and mitotic index (M.I.) in chickpea seeds. The 20 seeds per treatment were incubated for 15, 30, 45 and 60 minutes in filtered aqueous extracts (1, 3 and 5%). Un-treated seeds were used as negative control and 0.2% Ethylmethane sulphonate treated seeds as positive control. Chickpea seed germination and mitotic index exhibited highly significant variations at $p \leq 0.01$ (LSD) for most concentrations and their incubations for both tested plants. The inhibitory effects on germination were concentration and time dependent while mitotic index was mostly random for majority of treatment time and concentrations. Over all concentrations of R.S were less phytotoxic than L.R. as compare to +ve control.

Keywords: Incubation, Fertility enhancing plants, Germination, Mitotic index, Negative control, Positive control.

1. INTRODUCTION

Infertility affects reproductive system of men and women with almost equal frequency all over the world. The proportion of infertile population in Pakistan is 21.9% where, primary infertility is 3.9% and secondary infertility is 18%. Lots of peoples face many problems because of this state specially ladies face threats even divorced because of childlessness (Ali *et al.*, 2011). More than 80% of rural areas of Sindh depend on herbal remedies to cure disease (Rehman *et al*., 2011). Traditional plants used for infertility and its causes are mostly administered for long terms. Recent investigations have also revealed the presence of cytotoxic, compounds in many plants based traditional medicines including fertility booster plants (Yumnamcha *et al.,* 2014). Sometimes long term utilization of theses plant as complementary medicine for fertility enhancement may damage the healthy cell by attaching cellular membranes resulting in altered cell physiology and epigenetic changes to heredity material after nuclear membrane oxidation. Radish seeds and Rhizome of Sufed musli are most frequently used fertility enhancers plant parts in Sindh Pakistan **Radish** (*Raphanus sativus*) locally known as Mooli belongs to family brasicaceae. Radish seeds are very effective to treat infertility of both men and women. It basically keeps away from the risk of fatal malformation (healthyadvice.com) like severe oligospermia and less number of spermatozoa. Some highly bioactive secondary metabolites found in seeds of radish are glucosinolates (mustard oil glycosides), isothayociante, gluconasturtin, and sinigrin (Gutierrez and Perez, 2004).

Phytotoxic effects like inhibition of germination, root and hypocotyl length, fresh and dry matter weight by *Brassica* spp. may be caused by hydrolyed products of glucosinolates that occur in substantial amounts in the vegetative parts of *Brassica* spp (Jafariehyazdi and Javidfar, 2011) . Accumulation of heavy metals like Cadmium, copper, lead and zinc are also reported in radish (Saurbeck, 1991). Phytotoxic effects of different heavy metals concentration on seed germination and seedling growth in various crops: *Daucus carrota* (L.), *Raphanus sativus* (L.), Beta *vulgaris* (L.), *Lycopersium esculentum* (L.) and *Solanum melongena* (L.), *Vigna radiata* (L.), *Vigna angularis* (L.), *Lablab purpureus* (L.), *Lathyrus ordoratus* (L.), *Triticum aestivum* (L.), etc. were reported (Ilyin and Syso 2001; Azimi *et al*. 2006; Valerio *et al*. 2007, , Mantorova, 2010).

Plants containing high levels of Cd and Zink causes root growth inhibition, browning of roots , stunning and even leads to death by inhibiting enzyme activity of plant roots (Long *et al*., 2003; Mohanpuria *et al*., 2007; Versieren *et al*., 2017; Guo *et al*., 2018).

Land-calotrops (*Chlorophytum borivillianum*) locally called Sufed musli belongs to family liliaceae. Rhizome of Sufed musli posse's immunomodulatory properties and are used to cure impotency, sterility and enhance

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male potency (Khanam *et al.*, 2013). Rhizome of this plant contains higher saponin content; these are used as stimulants and metabolic enhancers. *Chlorophytum* contains number of bioactive compounds viz. over 25 alkaloids, steroids and saponins (Agrawal *et al.*, 2013). Steroids and alkaloids are reported mito-depressent in plant (Lee *et al*., 2014).

Phytotoxic history of these plants makes their utilization as alternative medicine questionable for long term. Therefore current study was done on two fertility booster plant parts (Radish seeds and roots of Land - calotrops) to predict non-cytotoxic effects in plant assay for being cheap and easy to handle and similarity of cellular sensitivity to plant bioactive compounds as suggested by many researchers.

2. MATERIALS AND METHODS

Concentrations and incubations of fertility enhancing plants: Seeds were incubated for 15, 30, 45 and 60 minutes in 1%, 3% and 5% solutions of aqueous extract of fertility enhancing plants for phytotoxicity and genotoxicity.

Preparation of aqueous extracts: Plant parts were collected and cleaned with cotton cloth to remove dust particles than powder was obtained by pestle and mortar and electric grinder **(Fig.1).** Powder of all the plants were soaked in distilled water for overnight followed by filtration with filter paper (Whatman No.1(12.5cm)).Un treated seeds were used as negative control and 0.2% EMS (Ethyl methane sulphonate) treated seeds as positive control **(Fig. 2).**

Incubation of seeds: 80 seeds per plant filtrate were placed in different concentrations and 20 seed per treatment were removed after 15, 30, 45 and 60 minutes. Before sowing seeds were washed with distilled water to stop the effect of plant extracts.

Germination: Incubated chickpea seeds were placed in sand pot up to 2 inches depth at 25C^{o} in green house for germination analysis. After 48 hours seeds were counted for root emergence (germination), procedure repeated until germination stopped **(Fig. 3).**

Fixation of mitotic roots: 2-2.5cm roots were systematically seek and placed in fixative (3:1 glacial acetic acid and alcohol) to determine cell division phase for one day then moved in 70% alcohol until slide preparation (Fig. 4).

Slide preparation and photography: Cut root tip very carefully and spread by squash technique (Dille and King 1983; Dille *et al.*, 1986), slides were stained with acetocarmine solution (2% acetocarmine in 45% glacial acetic acid), cover with Petri dish for 15 to 20 minutes for better staining, remove extra stain with filter paper and fix slides with flame.Slides were examined at 400 magnifications with the help of digital microscope (Olympus51x) **(Fig. 4).**

Cytogenetic studies: 6 slides per treatment were used to count initially normal dividing, abnormal dividing and non-dividing cells that were used to calculate mitotic index and abnormality index.

Fig. 1: Part of Plants and Grinding

Fig. 2: Extract preparation

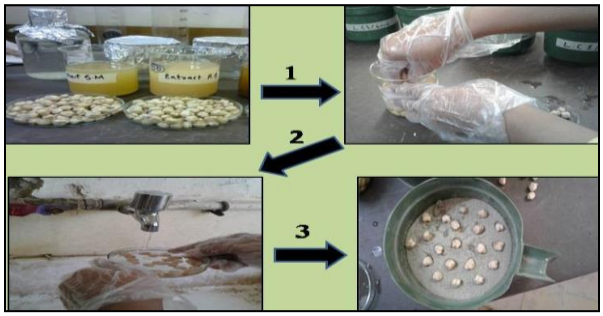

Fig. 3: Incubation and sowing of Chickpea seeds

Fig. 4: Storage of roots and Slide preparation

Data analysis of phytotoxicity

Germination percentage: To obtain germination percentage following formula used:

$$\text{Germinatio n\%} = \frac{\text{No. of germinated seed}}{\text{Total no. of seed sown}} \times 100$$

To compare highest and lowest rate in germination as compare to positive and negative control and to measure variability between the treatments means of mitotic index and abnormality index Least Square Difference (LSD) test at $p \leq 0.01$ was applied through computer software statistics 8.1.

Mitotic Index (M.I): A ratio between the numbers of total dividing cells and total cells analyzed called mitotic index. It was calculated by following formula.

$$\text{M.I.} = \frac{\text{Total dividing cells}}{\text{Total cells analyzed}} \times 100$$

3. RESULTS AND DISCUSSIONS

Germination (%): LSD analysis of chickpea seed germination (%) exhibited significant variations at $p \leq 0.01$ for all the doses of tested fertility enhancing plants.

Radish Seed and Land-calotrops aqueous extracts showed highly significant variation at $p \leq 0.01$ in most of incubations except 1% of both plants as compare to negative control.

Mean comparison of germination percentage:

The mean comparison of germination percentage of Radish Seed aqueous extract is presented in **(Table- 1).** Overall highest percentage of germination (85%) was recorded in 15 and 45 minutes incubation of 1% extract treatment and in 30 minutes of 3% treatment. Lowest germination (45%) was recorded in 45 minutes of 3% treatment **(Fig. 5).**

The mean comparison of germination percentage of Land-calotrops aqueous extract is presented in **(Table-2).** Maximum germination (90%) was recorded in 15 minutes incubation of 3%, minimum germination (50%) was recorded in 45 minutes incubation of 1% treatment **(Fig. 6).**

Mitotic Index:

Mitotic index is very important phytotoxicity parameter that provide solid prove of growth retardation. Therefore M.I. was calculated with help of number of non-dividing, normal dividing and abnormal dividing cells of Radish seed and Land-calotrops root **(Table 3 and 4).** Data was converted in M.I with statistically significant ($p \geq 0.01$) were found in all the doses of all plants aqueous extract for mitotic index in **(Table-5).**

Among tested concentrations highly significant variation in the M.I. of Radish seed was recorded in 1%, and 5% as compare to negative control. Maximum M.I. (92.45%) was observed in 3% and minimum (42.03%) in 1%. Rate of mitotic depression induced by 1% of radish seed was high between three concentrations. 5% showed ¾ time dependent mitotic depression **(Fig. 7).**

In mitotic index of Land-calotrops highly significant variation was observed in all periods of 5% whereas non-significant variation was observed in half of the 3 and 1% incubations as compare to controls. Maximum mitotic index (89.39%) given by 3% concentration incubation and minimum M.I. (77.48%) was observed in 5% **(Fig. 8).** Observed mitotic depression by Land-calotrops was inversely proportional to time and concentration incubations. 5% of Radish seed inhibit time dependent germination percentage whereas all concentrations of Land-calotrops randomly affect on germination percentage of chickpea seed.

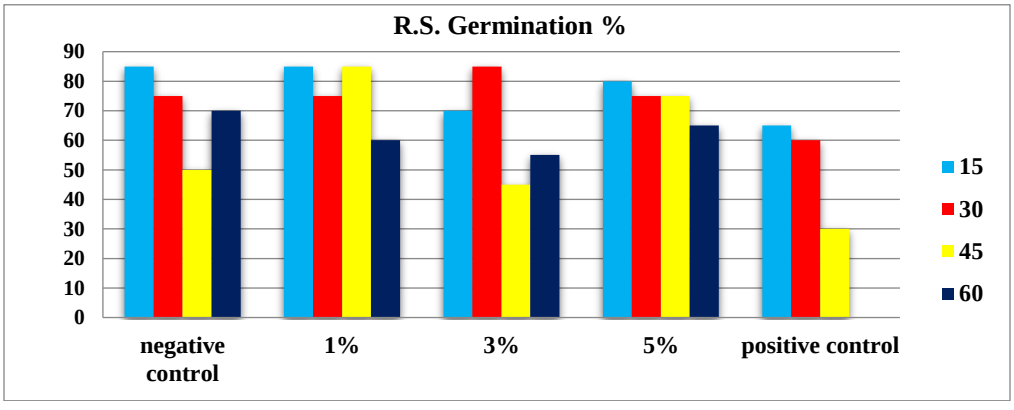

Fig. 5: Effect of different concentration of Radish (Seed) extracts on germination (%)

Table 1: Mean germination percentage of chickpea seeds treated by Radish (Seed) aqueous extracts (LSD p<0.01)

Treatment	Incubation (Minutes)	Number of cells		
		Non-dividing	Normal dividing	Abnormal dividing
-ve control	15	0	485	5
	30	0	480	10
	45	0	485	08
	60	0	490	0
R.S. 1 %	15	19	4	64
	30	23	2	34
	45	33	5	35
	60	142	8	180
R.S. 3%	15	38	6	354
	30	29	5	376
	45	50	10	226
	60	63	2	371
R.S. 5%	15	50	3	132
	30	95	3	161
	45	181	4	181
	60	161	2	181
EMS .2 % (positive control)	15	160	48	550
	30	180	30	468
	45	130	20	393
	60	-	-	-

Table 2: Mean germination percentage of chickpea seeds treated by Land-calotrops (Root) aqueous extracts (LSD p≤0.01)

S. No	Incubation Time (minutes)	-tive control	+tive control EMS (.2%)	Radish Seed (Aqueous extract)		
				1%	3%	5%
1	15	85^{a}	65^{e}	85^{a}	70^{d}	80^{b}
2	30	75^{c}	60^{f}	75^{c}	85^{a}	75^{c}
3	45	50^{h}	30^{j}	85^{a}	45^{i}	75^{c}
4	60	70^{d}	00^{k}	60^{f}	55^{g}	65^{e}

(Means with same alphabets are non-significantly different from each other and with different alphabets are significantly different at (p≤0.01)

Table 3: Number of normal dividing, non-dividing and abnormal dividing cells induced by different concentration of Radish (seed)

S. No	Incubation Time (minutes)	-tive control	+tive control EMS (.2%)	Land Calotrops (Aqueous extract)		
				1%	3%	5%
1	15	85^{b}	65^{f}	75^{d}	90^{a}	75^{d}
2	30	75^{d}	60^{g}	65^{f}	75^{d}	60^{g}
3	45	50^{i}	30^{j}	50^{i}	80^{c}	80^{c}
4	60	70^{e}	00^{k}	65^{f}	55^{h}	60^{g}

(Means with same alphabets are non-significantly different from each other and with different alphabets are significantly different at (p≤0.01)

Table 4: Number of normal dividing, non-dividing and abnormal dividing cells induced by different concentration of Land-calotrops (Root)

Treatment	Incubation (Minutes)	Number of cells		
		Non-dividing	Normal dividing	Abnormal dividing
-ve control	**15**	0	485	5
	30	0	480	10
	45	0	485	08
	60	0	490	0
L.R. 1 %	**15**	21	3	229
	30	49	5	222
	45	58	3	227
	60	68	3	281
L.R. 3%	**15**	30	2	251
	30	41	4	228
	45	46	8	330
	60	61	3	270
L.R. 5%	**15**	41	3	210
	30	41	5	248
	45	52	4	175
	60	77	3	294
EMS .2 % (positive control)	**15**	160	48	550
	30	180	30	468
	45	130	20	393
	60	-	-	-

Table 5: Mitotic index of chick pea root tip cells incubated by different concentrations and durations of Radish seed and Land-calotrops (root)

Type of index	Duration (mints)	-ve Cont	+ve cont	Concentration		
				1%	3%	5%
Mitotic index of Radish seed	**15**	100^{a}	82.25^{e}	78.16^{f}	90.45^{c}	72.97^{h}
	30	100^{a}	76.32^{g}	61.01^{j}	92.25^{b}	63.32^{i}
	45	100^{a}	73.35^{h}	54.79^{l}	82.51^{e}	50.54^{m}
	60	100^{a}	$-^{n}$	56.96^{k}	85.55^{d}	53.19^{l}
Mitotic index of Land- calotrops root	**15**	100^{a}	82.25^{f}	86.56^{c}	89.39^{b}	83.85^{de}
	30	100^{a}	76.32^{h}	82.27^{f}	84.98^{d}	84.33^{d}
	45	100^{a}	73.35^{i}	79.86^{g}	88.02^{bc}	77.48^{h}
	60	100^{a}	$-^{j}$	80.68^{g}	81.73^{fg}	79.41^{g}

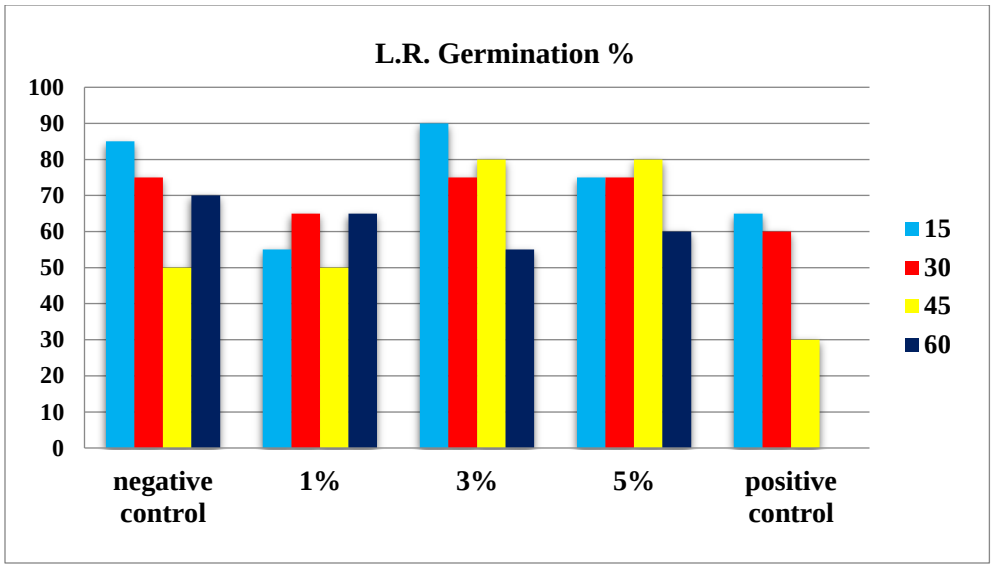

Fig. 6: Effect of different concentration of Land-calotrops (Root) extracts on germination (%)

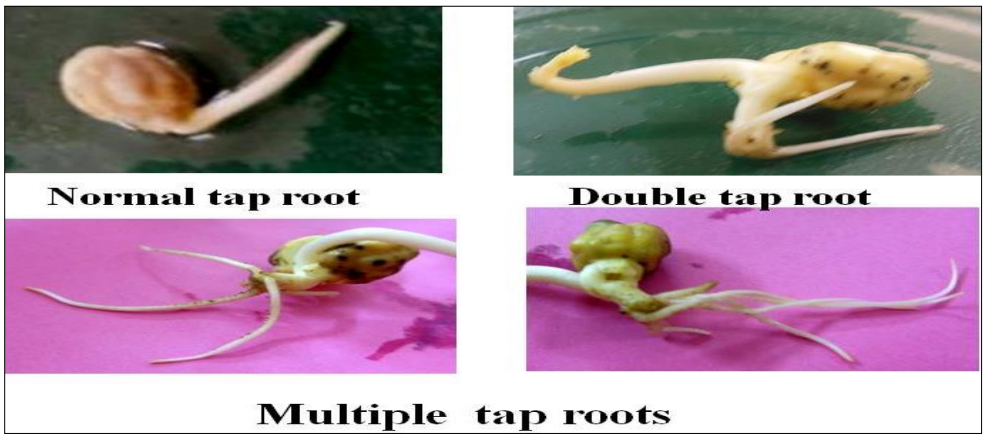

Fig. 7: Tap root mutation induced by fertility enhancing plants

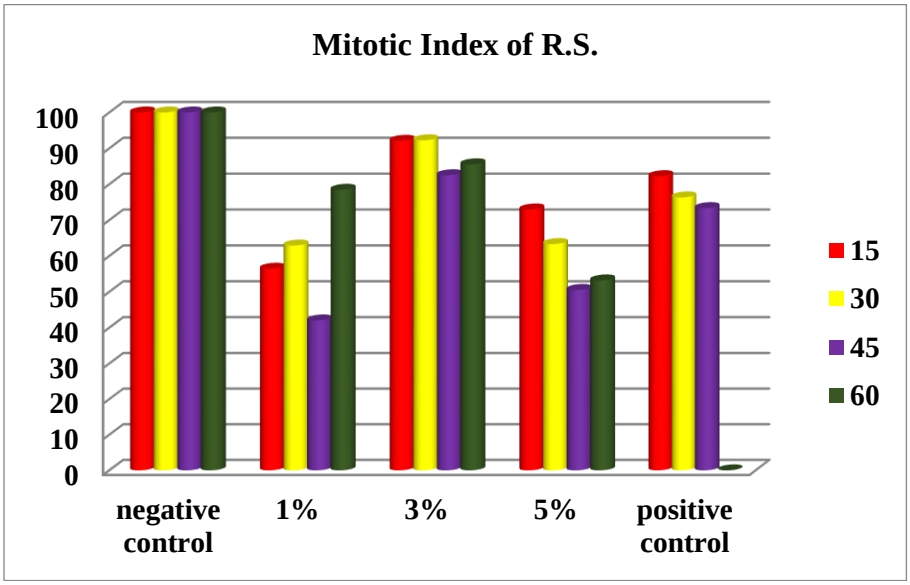

Fig. 8: Mitotic Index given by different concentration and incubation of Radish (Seed)

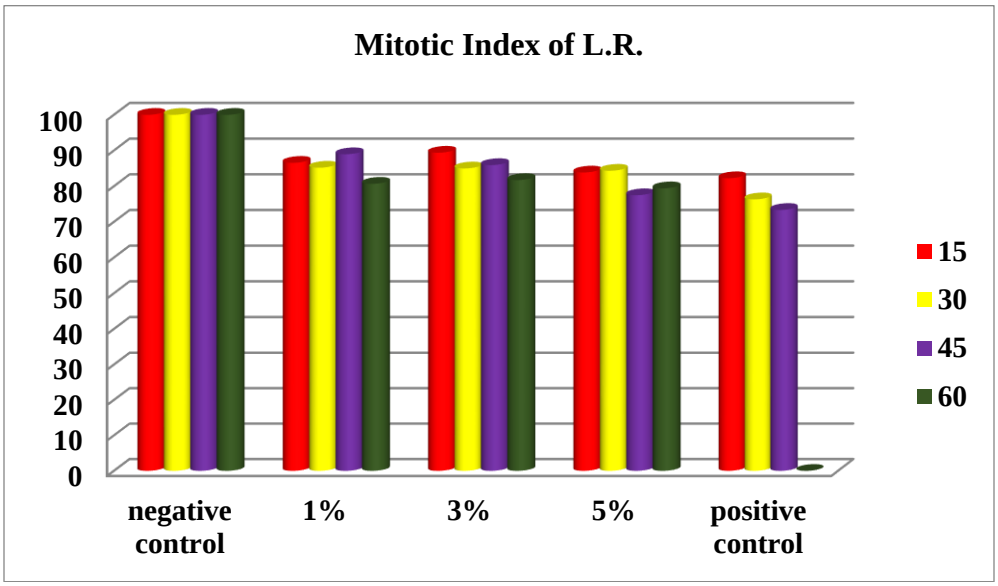

Fig. 9: Mitotic Index given by different concentration and incubation of Land-calotrops root

On the contrary other scientists found concentration dependent germination inhibition as well as yield and growth inhibition mediated by different plant extracts. Eucalyptus gum and leaf, Moringa, prickly Chaff-flower, Thyme seed, Neem leaf and seed, Radish aerial part and root, stem and leaves of milk weed were reported to cause concentration dependent decline in Maize, Moong bean, Pear millet, Sorghum, Guar, Chickpea, beans and corn seed germination and yield of wheat respectively (Bogatek *et al.*, 2006; Khan *et al.*, 2007; Hossain *et al.*, 2012; Tanveer *et al.*, 2014, Qureshi *et al.*, 2015, Gomes *et al.*, 2017 and Shah *et al.*, 2017). This may be due to high differences in applied concentrations in these experiments i-e. 2.5, 5, 10 and 20%. Similar findings were recorded for *Satureja thymbra* L. and *Cassia fistula* extracts in different assay plants by various researchers in near past (Masoud, 2018 and Muhammad, 2019) . Whereas other incubations of rest of plants revealed that chick pea seeds were totally compromised irrespective used aqueous extracts. Currents findings are although rare but occasionally reported from Brazil (Gomes *et al.*, 2017). Moderately toxic plants given concentration dependent toxicity, whereas highly toxic plants by plant aqueous extracts given random effect for germination inhibition. As metals are associated with many physiological and biochemical disorders (Ackova, 2018) therefore observed germination inhibition can be correlated with noticeable heavy metals accumulation in Radish seed (Cadmium, copper, lead and zinc) (Saurbeck, 1991) and Land-calotrops (Arsenic, Lead, cadmium, Mercury, chromium and Nickel) as reported earlier (Behera and Bhattacharya, 2016), Inhibition of seed germination and plant root growth retardation by cadmium, lead and arsenic tested in chick pea and onion is reported from many researchers around the world (Babatunde and Bakare, 2006; Mondal *et al*., 2013; Bhattacharya *et al.*, 2012). Reduction in wheat seed length, germination inhibition and membrane leakage in pea seed and zea mays root length and germination inhibition as well root growth inhibition induced by high efficacy of zinc, iron, cadmium and mercury (Rasafi *et al*., 2016, Pattanaik *et al*., 2011 and Rahoui, *et al*., 2010).

Incubation dependent decrease in mitotic index was observed by many researchers in aqueous extracts of *Vicia villosa* , *Rubus sancatus*, *Cinnamomum zeylanicum* (bark) and *Citrullus colocynthis* (leaves) by using *Allium cepa* and *Viccia faba* root tip cells (Soltys *et al*., 2011; Selmi *et al*., 2014; El-Ghamery and Basuoni, 2015; Sameer, 2016). The ethanol extract of *Peganum harmala* also induced similar effects on *Viciafaba* root meristem (Mekki, 2014). Time & dose dependent decrease in mitotic index was also found from herbicides (Agil & flurochloridone) and pesticide (malathion) based on *Allium cepa* assay (Yuzbasioglu *et al*., 2003; Elena, 2012; Singh and Roy, 2017). Time dependent decrease and fluctuated curve may be due to random effect of phytochemical glucosinolates. Metabolites of glucosinolates (thiocyanates, thiourea and oxazolidithione) are liable to disturb thyroid function, act as causative agents of goitrogenicity, mutagenicity, hepatotoxicity and nephrotoxicity (Ahlin *et al*., 1994; Zang *et al*., 1999; Wallig *et al*., 2002 and Tanii *et al*., 2004). In addition to mitodepressive effects in plants by heavy metals, earlier researchers also notified human toxicity by the presence of lead and cadmium results liver damage (cytotoxicity) increased

blood enzyme levels and reduced protein synthesis (both are molecular indicator of oncogenesis) (El-Boshy *et al.,* 2017; Yuan *et al*., 2014).

As observed by earlier reporters, in root tip cell of onion mitotic index inhibition produce by water samples in Malaysia and *Aloe vera* gel extract in Turkey (Akinoboro *et al*., 2011; Ilbas *et al*., 2012). Non dose dependent mitotic depressive effects of Land-calotrops related with alkaloids in the presence of other chemicals as steroidal belongs to family liliaceae are dangerous for animals, responsible for teratogenicity resulting in craniofacial birth defects in lamb (Lee *et al*., 2014). Plant chemicals like titerpenoids, phenolic compounds and flavonoids acting as anticarcinogens, when used in elevated level lower cell division of non targeted cells also (Akinboro *et al*., 2011; Bishayee *et al.*, 2011).

4. CONCLUSION

Both tested plants (Radish and Land-calotrops) were potentially phytotoxic to assay plant (Chickpea) with minor differences. Root germination percentage was more sensitive to land- calotrops aqueous extracts, whereas mitotic index to radish seeds. The observed high phytotoxicity alarms the possible cellular damage to human cells if administered prolonged.

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