ENHANCED RECLAMATION EFFICIENCY OF *Vetiveria zizanioides* AT POLLUTED SITES COMPARED TO EXPERIMENTAL PLOTS

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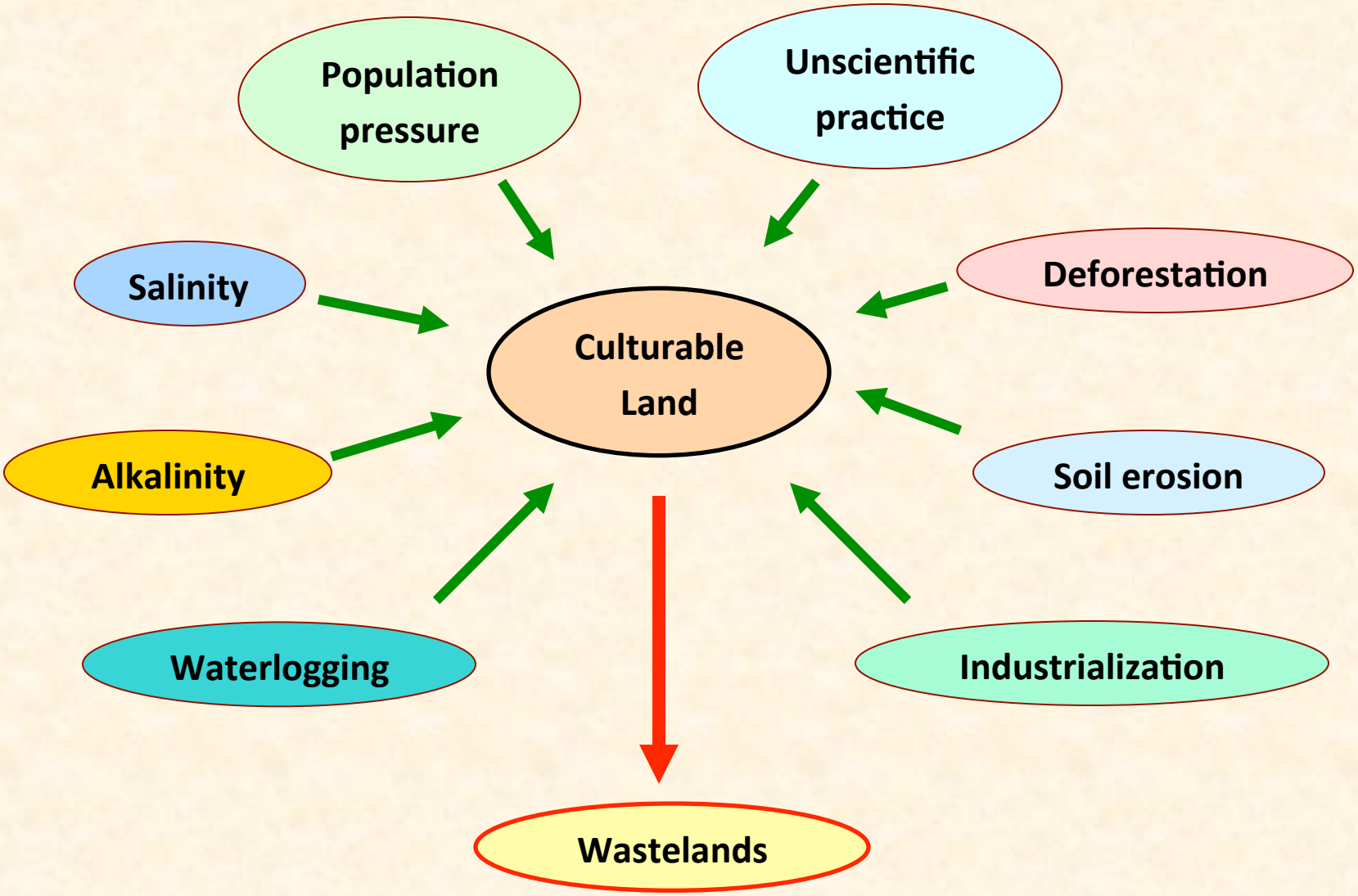

Population pressure
Unscientific practice
Salinity
Deforestation
Culturable Land
Alkalinity
Soil erosion
Waterlogging
Industrialization
Wastelands

Classification of Wastelands

Wastelands

Culturable Wastelands

have ability to develop vegetation cover and not being used due to different constraints of varying degree

Examples:

- Gullied lands
- Waterlogged lands
- Mining lands
- Industrial wastelands
- Grazing lands
- Degraded forestlands
- Salt affected lands
- Marshy lands
- Strip lands etc.

Unculturable Wastelands

not able to develop the vegetative cover

Examples:

- Barren rocky
- Sheet-rock area
- Steep-sloping area
- Snow-covered area
- Glacial area etc.

According to 'National Wastelands Development Board'

Details of wastelands in India as per (National Remote Sensing Agency) estimates

Types	Details	Area in m.ha.
Culturable Wastelands	Saline	3.9
	Gullied and Ravinous	4.32
	Water logged	0.89
	Undulating uplands with or without Scrub	10.79
	Jhum Cultivation and Forest Blanks	2.4
	Sandy Areas	10.53
	Total	32.83
Unculturable Wastelands	Barren Hills and Rock out-Crops	2.75
	Snow Bound Area	17.7
	Total	20.45
Grand Total		53.28

About 53.28 million hectare (17.45% of total geographical area) exist in India till date. Uttar Pradesh possesses 2.29 million hectare wastelands, which accounts for approximately 4.14% of total wastelands in India.

(Source: 1:50,000 scale wasteland maps-1986-2000 prepared based on Landsat Thematic Mapper/IRS-LISS II and map-2003 prepared based on IRS-LISS III data)

Characteristics of species used for reclamation

- Accumulate the nutrients
- Change the structure of soil
- Mitigate the toxicity levels of soil
- Have deep and large root system
- Be fast growing
- Require least input and negligible attention,
- Grow under variety of environmental conditions, etc.
- Besides, the species must be economically useful and should be able to give cash returns.

In this light some medicinally important plants have been the choice of the present investigation.

- **Plant chemicals- Primary metabolites,**
  Secondary metabolites
- **Plants respond to the stress conditions by accumulating the secondary metabolites**
- **The conditions of wastelands are very harsh and stressful**
- **Plant tissue cultures could be used for production of bioactive plant metabolites (Rao and Ravishankar, 2002) and even help in commercial processing of rare plants (Patwardhan, *et al.*, 2004)**
- **The cultivation of medicinal plants on wastelands may further be able to decrease the pressure on wild medicinal plants utilized by local people protecting them from being endangered.**

Important aspects of present investigation

- Selection of 'culturable degraded land' sites.
- Survey of plants growing on the sites and selection of plants likely to survive on sites and be useful for land reclamation and medicinal use
- Study of soil and plants at different intervals to estimate gain or loss to the soil and to the selected plants on sites compared to controls (nursery grown)
- Study of status of medicinal principle of selected plants on sites compared to controls
- Using explant from the plants growing on degraded land sites and nursery site, calli raised and their medicinal principle compared for added advantage, if any

Objective

- To find out the right stage of plantlet for best results with respect to soil reclamation and yield of plant products.
- To find out the effect of Vetiver / contaminated site, on the medicinal plant grown as a side cash crop
- The advantage of Vetiver over the wild plants growing on the polluted sites

Selected Sites

List of plants growing wild at selected sites

On AEE site	On DSS site	On BEE site
Rumex	*Rumex*	*Rumex*
Cannabis	*Achyranthes*	*Cannabis*
Ricinus	*Malvastrum*	*Ricinus*
Parthenium	*Parthenium*	*Parthenium*
Cyanodon	*Cyanodon*	*Calotropis*
Phalaris	*Phalaris*	*Phalaris*
Croton	*Solanum species*	*Solanum species*
Coriandrum	*Stellaria*	*Commelina*
Tagetes	*Argemone*	*Coronopus*
	Dalbergia	*Chenopodium*
	Cassia	*Withania*
	Eclipta	*Silene*
	Ranunculus	*Anagalis*
	Melilotus	*Melilotus*
	Senebiera	*Amaranthus*
	Lindenbergia	*Abutilon*

***Vetiveria zizanioides* (L.) Nash.**

Chemical constituents:

Sesquiterpene and their derivatives

khusimol, khusinol, khusimone, α-vetivone, β-vetivone, junipene, γ-cadenene, clovene, α-amorphine, aromadenrine, epiglobulol, spathulenol etc.

Medicinal Uses:

- Prostrate cancer, diabetes, hernia, stomach problems, skin disease, irritations, kidney stones etc.
- has abortifacient, antioxidant, anti-carcinogenic, termicidal, Insect repellent properties.
- Also used in aromatherapy

Withania somnifera (L.) Dunal.

Chemical constituents:

Alkaloids-

Nicotine, Tropine, Pseudotropine, Anaferine, Withanine, Somniferine, Somniferinine, Somnine, Pseudowithanine, Withananine, Withananinine, Isopelletirine, Anahygsine

Withasteroids (steroidal lactone)-

Withanolide A, Withanolide E, Withanolide D, Withaferine A, Withanone, 27-hydroxy Withanone,

Medicinal Uses:

Impotency, fever, ulcers, cough, typhoid, rheumatism, leukoderma, hiccup, anxiety, depression, anti-aging, leucorrhoea, diarrhoea, dropsy, hypertension, menstrual troubles, uterosis, dyspepsia, Immunorestorative, cardiovascular protection, osteoarthritis etc.

Being a medicinally popular plant and also because of its inclusion in the RET (Rare, Endangered and Threatened) species (Kavidra *et al.* 2000) this plant was selected for possible reclamation of degraded culturable soils with pharma farming

Methods

- Estimation of Sodium and Potassium (Pratt and Fathi-Ettai, 1990)
- Determination of Total Proteins (Bradford *et al.,* 1976)
- Determination of Reducing, Non-reducing and Total sugar (Nelson, 1952)
- Estimation of Total Phenolic content (Bray and Thorpe, 1954)
- Extraction procedure for total wihanolides (Gupta *et al.*, 1996)
- Cation Exchange Capacity (CEC) (Jones, 1967)
- Estimation of Total Nitrogen (Snell and Snell, 1967)
- Estimation of Organic Carbon (Datta *et al.,* 1962)
- Estimation of phosphorus content (Olsen, 1954)
- Estimation of Biological oxygen demand (BOD) (Winkler's iodometric Method)

Experimental plan

Selection of sites-

Experimental design-

- **Pot filling**
- **Planting of 4 leaf, 5 leaf and 6 leaf plantlets of *V. zizanioides* in pots**
- **Morphological study of pot plants**
- **Biochemical study of soil and leaf of pot plants at regular intervals of one month upto six month and finally after 12 months**
- **Planting of 5 leaf stage plantlets on different selected sites (in fields)**
- **Biochemical study of soil and leaf of field plants at regular intervals of 3 months**
- **Planting of one month old seedlings of *Withania somnifera* in pots and on fields of selected soil provenances**
- **Biochemical study of soil and plants (pot and field) after regular intervals of 3 months**
- **Callus induction and culture for secondary metabolites and for regeneration purpose using explants from the plants grown in pots containing soil of selected provenances**

Vetiver pot and field plantation

Five months old pot plantation

12 months old field plantation

Fig.10 % rise or fall in OC in potted soils of different provenances during 0-5 months of *V. zizanioides* 4, 5 and 6-leaf plantlet growth

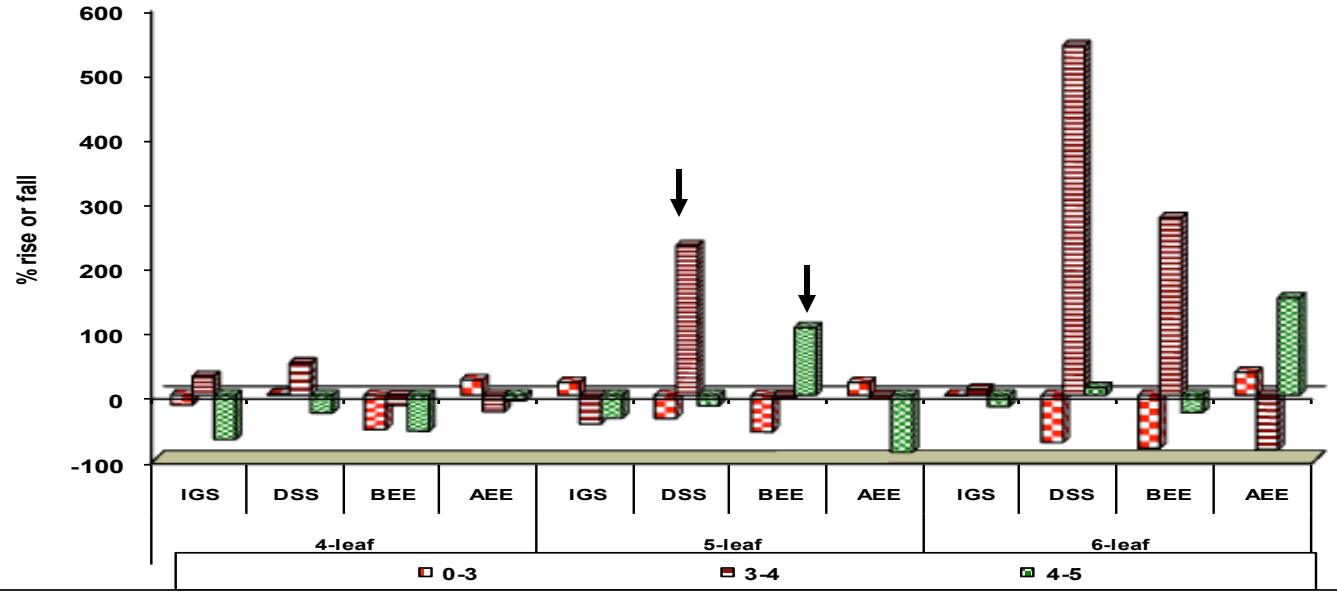


Fig.11 % rise or fall in OC during 0-5 months in *V. zizanioides* 4, 5 and 6-leaf plantlet grown on soils of selected sites in pots

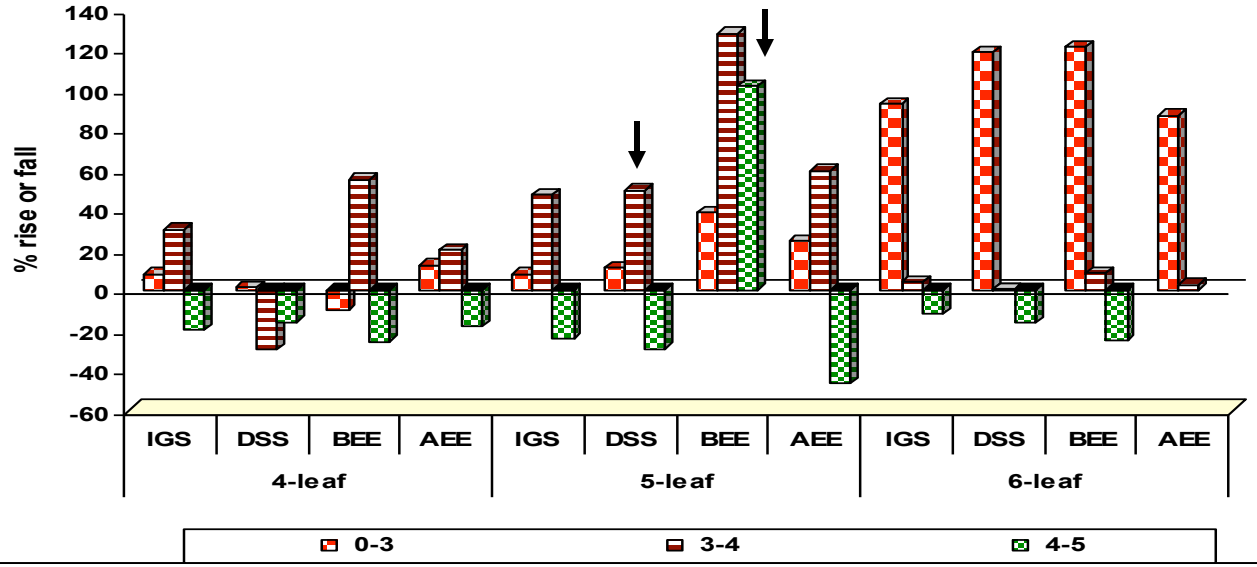


Fig.12 % rise or fall in nitrogen in potted soils of different provenances during 0-5 months of *V. zizanioides* 4, 5 and 6-leaf plantlet growth

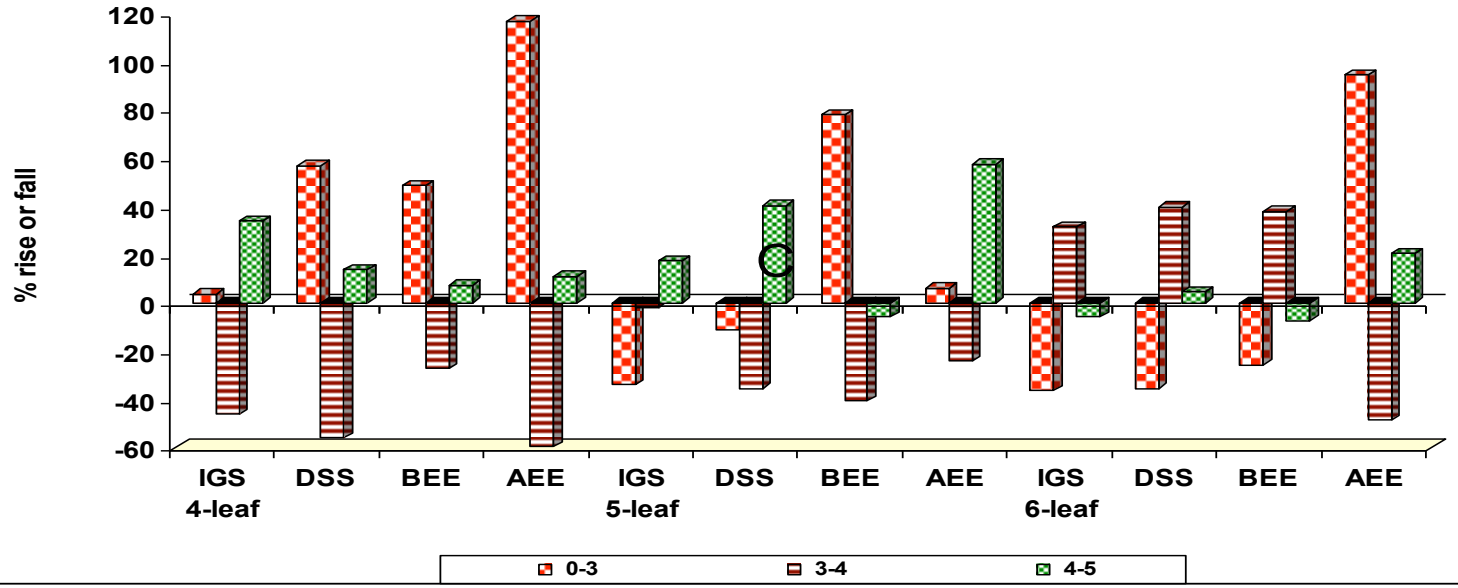


Fig.13 % rise or fall in nitrogen during 0-5 months in *V. zizanioides* 4, 5 and 6-leaf plantlet grown on soils of selected sites in pots

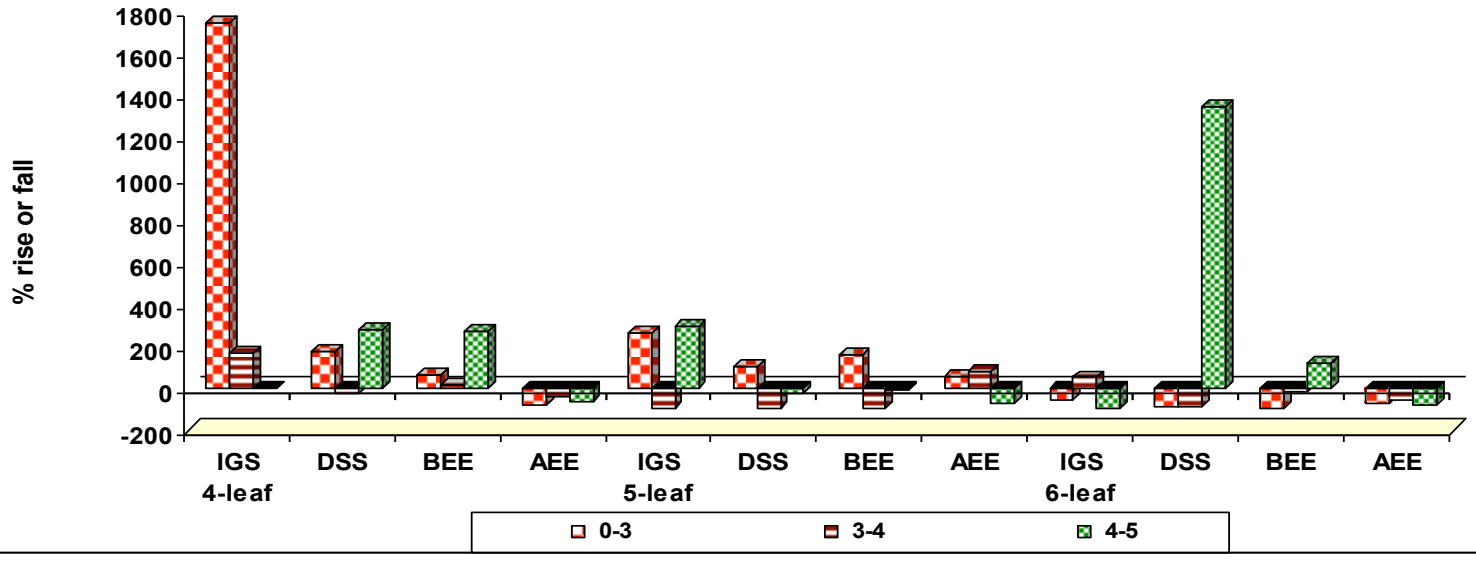


Fig. 46 Biochemical attributes of potted soil and plant (leaf) of selected provenances in *Vetiveria zizanioides*

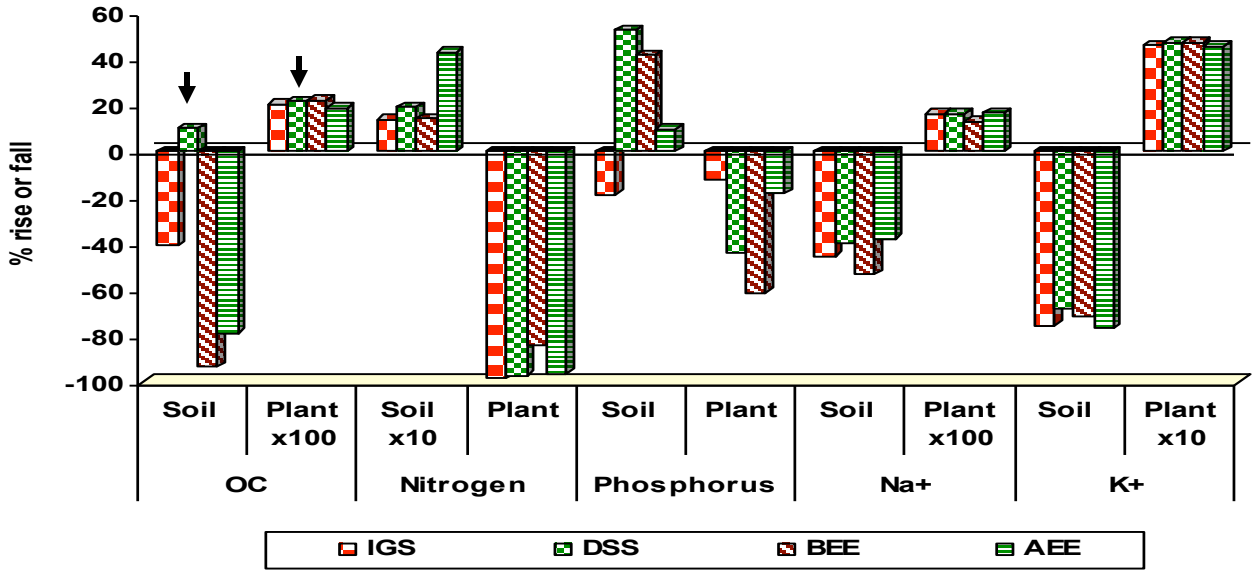
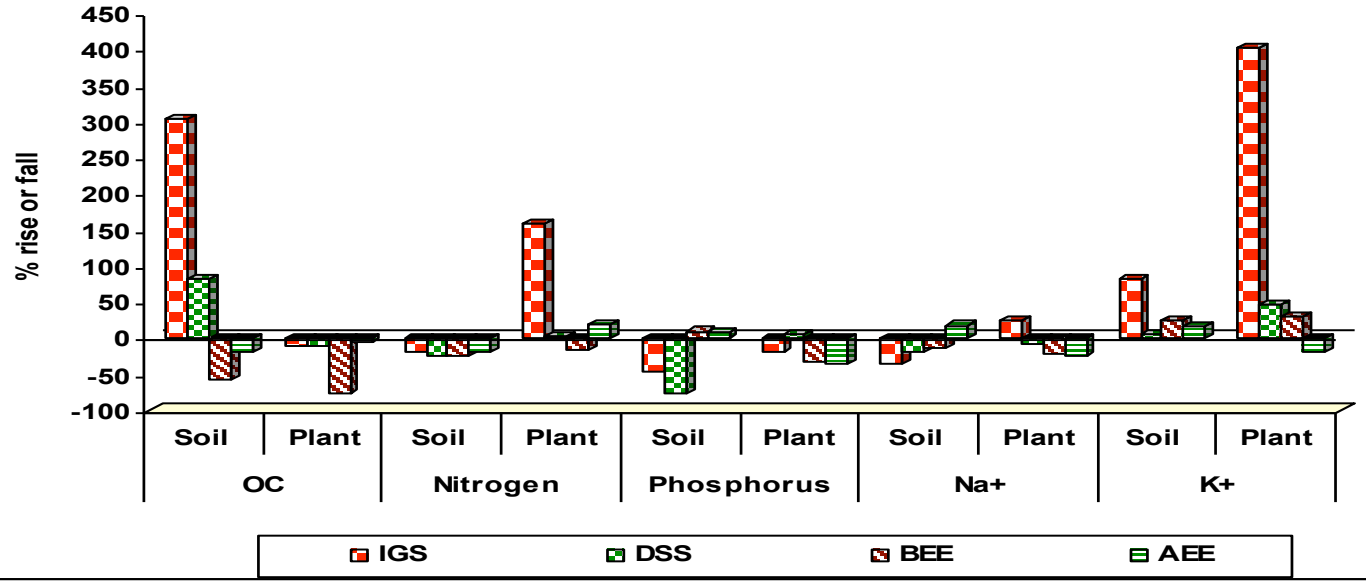


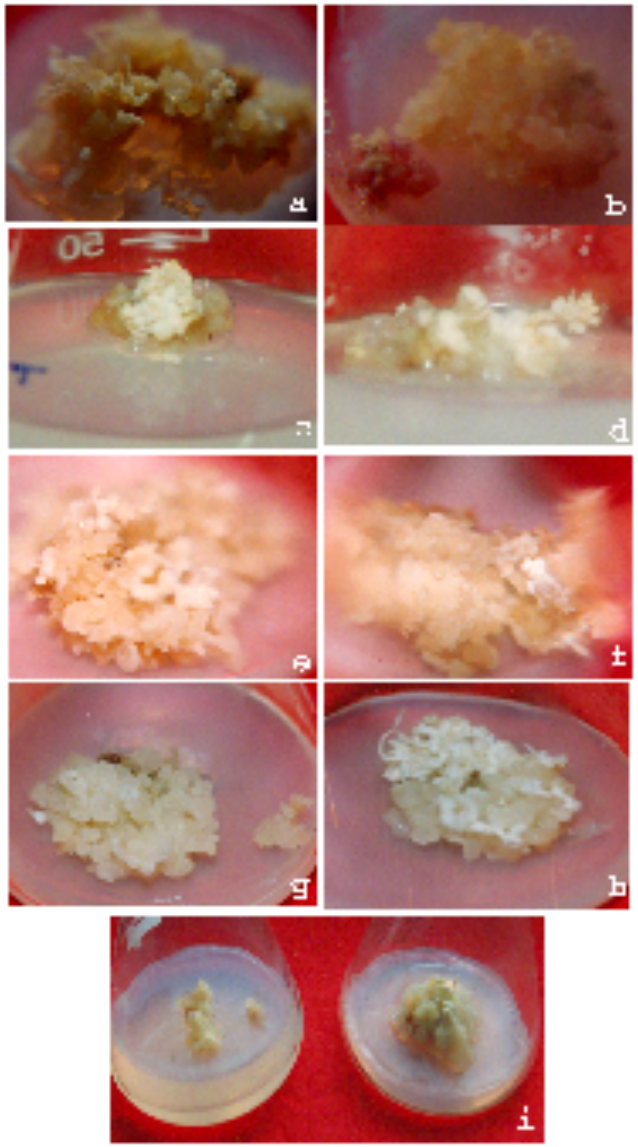
Fig.47 Biochemical attributes of field soil and plant (leaf) of selected soil provenances in *Vetiveria zizanioides*



Morphological response of *Vetiver zizanioides* on different media *in vitro*

Media Tested (MS+3% sucrose)	Transferred on (MS+)	Degree of response	Days of Response	Type of callus
0.5-2,4-D	–	+++	7	white & sticky
1.0-2,4-D	–	++	7	white & sticky
2.0-2,4-D+0.5 Kn	–	+	56	white
0.5-2,4-D	3% sucrose	No growth	–	black
1.0-2,4-D	3% sucrose	No growth	–	black
0.5-2,4-D	4% sucrose+0.5Kn	No growth	–	more black
1.0-2,4-D	4% sucrose+0.5Kn	No growth	–	more black
0.5-2,4-D	2%sucrose+25CM	+	20	less rooting
	2%sucrose+50CM	++	15	more rooting
	2%sucrose+100CM	+	20	less rooting
1.0-2,4-D	2%sucrose+25CM	+	20	less rooting
	2%sucrose+50CM	++	15	more rooting
	2%sucrose+100CM	+	20	less rooting
0.5-2,4-D	2%sucrose+2.0-2,4-D	No growth	–	–
	2%sucrose+4.0-2,4-D	No growth	–	–
1.0-2,4-D	2%sucrose+2.0-2,4-D	+	25	white
	2%sucrose+4.0-2,4-D	++	29	white
0.5-2,4-D	3% sucrose+2 BA	No growth	–	–
1.0-2,4-D	3% sucrose+2 BA	No growth	–	–
0.5-2,4-D	2% sucrose	+	23	white
1.0-2,4-D	2% sucrose	+	26	white

V. zizanioides culm-node Callus raised on different media



Rooting and anthocyanin production (MS +0.5mg/l 2,4-D)

10-week-old callus after 4th subculture

Different stages of calli transferred on different media for regeneration

Plantlet regeneration in *V. zizanioides*
(Explant taken from AEE grown potted plants)

Callus induction →

Plantlets regenerated →

Regenerated shoots →

3 months old

Withania somnifera
pot plantation

6 months old

12 months old pot plantation of *Withania somnifera*

Withania somnifera seedlings and 12-month-old field plantation on-

Ditch site soil (DSS)

Before effluent entry (BEE)

After effluent entry (AEE)

•Soil under Vetiver plant

Soil type	Comparison	pH	CEC meq./ 100gdwt	% Organic carbon	Nitrogen mg/gdwt	Phosphorus µg/gdwt	Na+ meq./gdwt	K+ meq./gdwt	Na+/K+ ratio
IGS	Initial	7.82	8.65	0.0000893	0.583	3.53	2.83	0.92	3.07
	6th	7.70	17.60	0.000703	0.468	2.08	2.09	1.29	1.62
	12th	7.77	18.92	0.00036	0.476	1.86	1.85	1.67	1.10
	6th vs initial	-1.53	103.47	687.23	-19.73	-41.08	-26.15	40.22	-47.23
	12th vs initial	-0.64	118.73	303.14	-18.35	-47.31	-34.63	81.52	-64.17
DSS	Initial	6.32	9.90	0.000288	0.563	3.32	2.62	1.12	2.33
	6th	6.85	13.86	0.000508	0.876	1.09	2.16	0.136	1.58
	12th	7.25	14.08	0.000523	0.423	0.768	2.09	1.19	1.75
	6th vs initial	8.39	40.00	76.39	55.60	-67.17	-17.56	-87.86	-32.19
	12th vs initial	14.72	42.22	81.60	-24.87	-76.87	-20.23	6.25	-24.89
BEE	Initial	6.40	21.78	0.00064	0.509	3.73	2.8	1.55	1.80
	6th	6.40	7.48	0.000711	1.467	1.32	2.37	0.18	1.32
	12th	6.57	10.56	0.000277	0.381	4.08	2.39	1.93	1.23
	6th vs initial	0.00	-65.66	11.09	188.21	-64.61	-15.36	-88.39	-26.67
	12th vs initial	2.66	-51.52	-56.72	-25.15	9.38	-14.64	24.52	-31.67
AEE	Initial	6.33	22.66	0.00075	0.529	2.81	2.67	1.25	2.13
	6th	6.65	17.38	0.00104	0.931	4.19	2.93	1.47	1.99
	12th	6.52	17.60	0.000607	0.425	3.05	2.2	1.45	1.51
	6th vs initial	5.06	-23.30	38.67	75.99	49.11	9.74	17.60	-6.57
	12th vs initial	3.00	-22.33	-19.07	-19.66	8.54	-17.60	16.00	-29.11

Soil type	Comparision	% Organic carbon	Protein mg/gfwt	Nitrogen mg/gdwt	Phosphorus µg/gdwt	Total sugars mg/gdwt	Reducing sugars mg/gdwt	Non-reducing sugars mg/gdwt	Na^+ meq./ gdwt	K^+ meq./ gdwt	Na^+/K^+ ratio
	Initial	0.018	5.29	0.112	11.24	0.222	0.215	0.007	155.08	46.03	3.36
IGS	6th	0.021	0.88	0.315	9.81	0.204	0.181	0.023	125.41	41.48	3.02
	12th	0.016	1.77	0.402	8.98	0.213	0.192	0.021	139.27	65.49	2.12
	6th vs initial	14.444	-83.365	181.250	-12.722	-8.108	-15.814	228.571	-19.132	-9.885	-10.119
	12th vs initial	-10.000	-66.541	258.929	-20.107	-4.054	-10.698	200.000	-10.195	42.277	-36.905
DSS	**Initial**	0.018	5.29	0.112	11.24	0.222	0.215	0.007	155.08	46.03	3.36
	6th	0.022	3.44	0.114	19.62	0.123	0.108	0.015	119.5	40.76	2.93
	12th	0.020	3.06	0.114	11.71	0.328	0.144	0.184	140.3	67.49	2.07
	6th vs initial	23.889	-34.972	1.786	74.555	-44.595	-49.767	114.286	-22.943	-11.449	-12.798
	12th vs initial	-0.018	-5.290	-0.112	-11.240	-0.222	-0.215	-0.007	-155.080	-46.030	-3.360
BEE	**Initial**	0.018	5.29	0.112	11.24	0.222	0.215	0.007	155.08	46.03	3.36
	6th	0.030	3.9	0.142	13.65	0.27	0.213	0.057	120.41	48.98	2.45
	12th	0.032	1.37	0.092	7.52	0.201	0.175	0.026	120.41	60.07	3.36
	6th vs initial	65.556	-26.276	26.786	21.441	21.622	-0.930	714.286	-22.356	6.409	-27.083
	12th vs initial	77.222	-74.102	-17.857	-33.096	-9.459	-18.605	271.429	-22.356	30.502	0.000
AEE	**Initial**	0.018	5.29	0.112	11.24	0.222	0.215	0.007	155.08	46.03	3.36
	6th	0.023	3.56	0.102	12.64	0.25	0.208	0.042	119.5	3039	3.93
	12th	0.017	1.32	0.132	7.28	0.308	0.145	0.163	114.95	36.82	3.12
	6th vs initial	27.778	-32.703	-8.929	12.456	12.613	-3.256	500.000	-22.943	-33.978	16.964
	12th vs initial	-5.000	-75.047	17.857	-35.231	38.739	-32.558	2228.571	-25.877	-20.009	-7.143

Fig.82 Biochemical attributes of potted soil and plant (leaf) of selected soil provenances in *Withania somnifera*

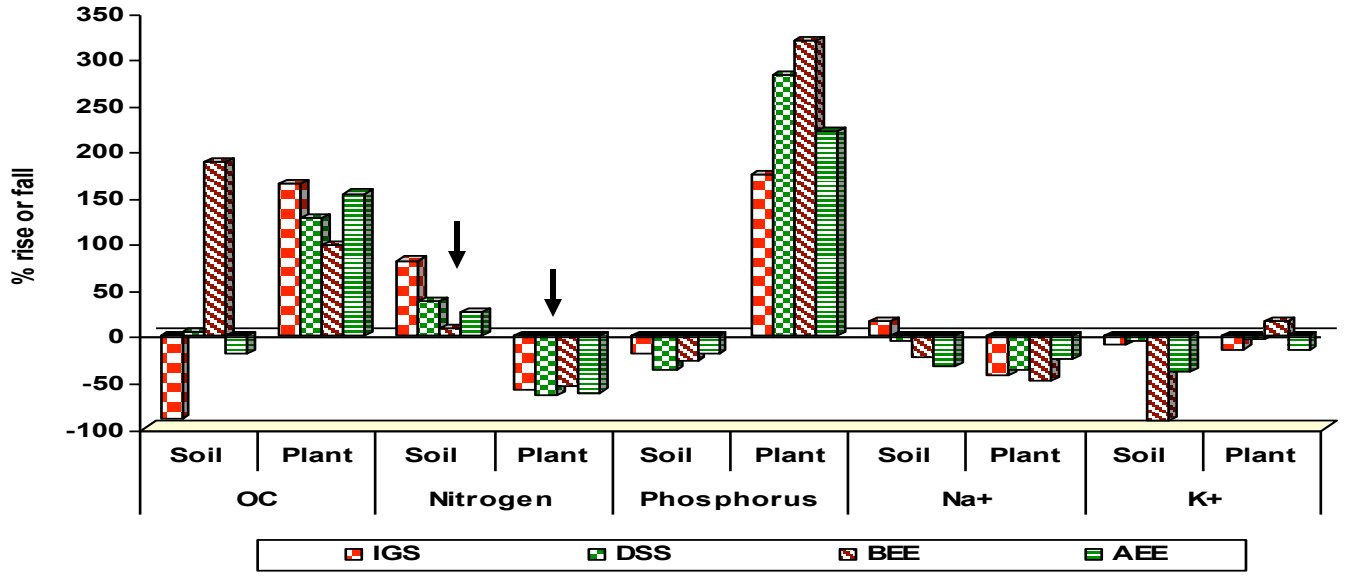


Fig.83 Biochemical attributes of field soil and plant (leaf) of selected soil provenances in *Withania somnifera*

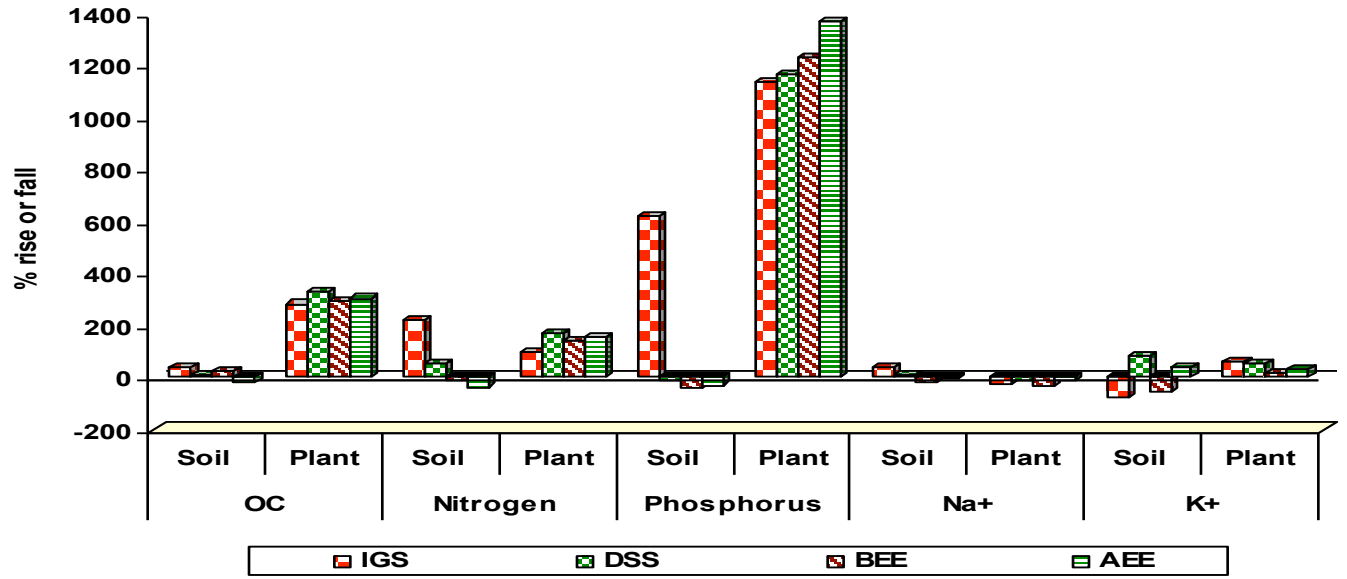
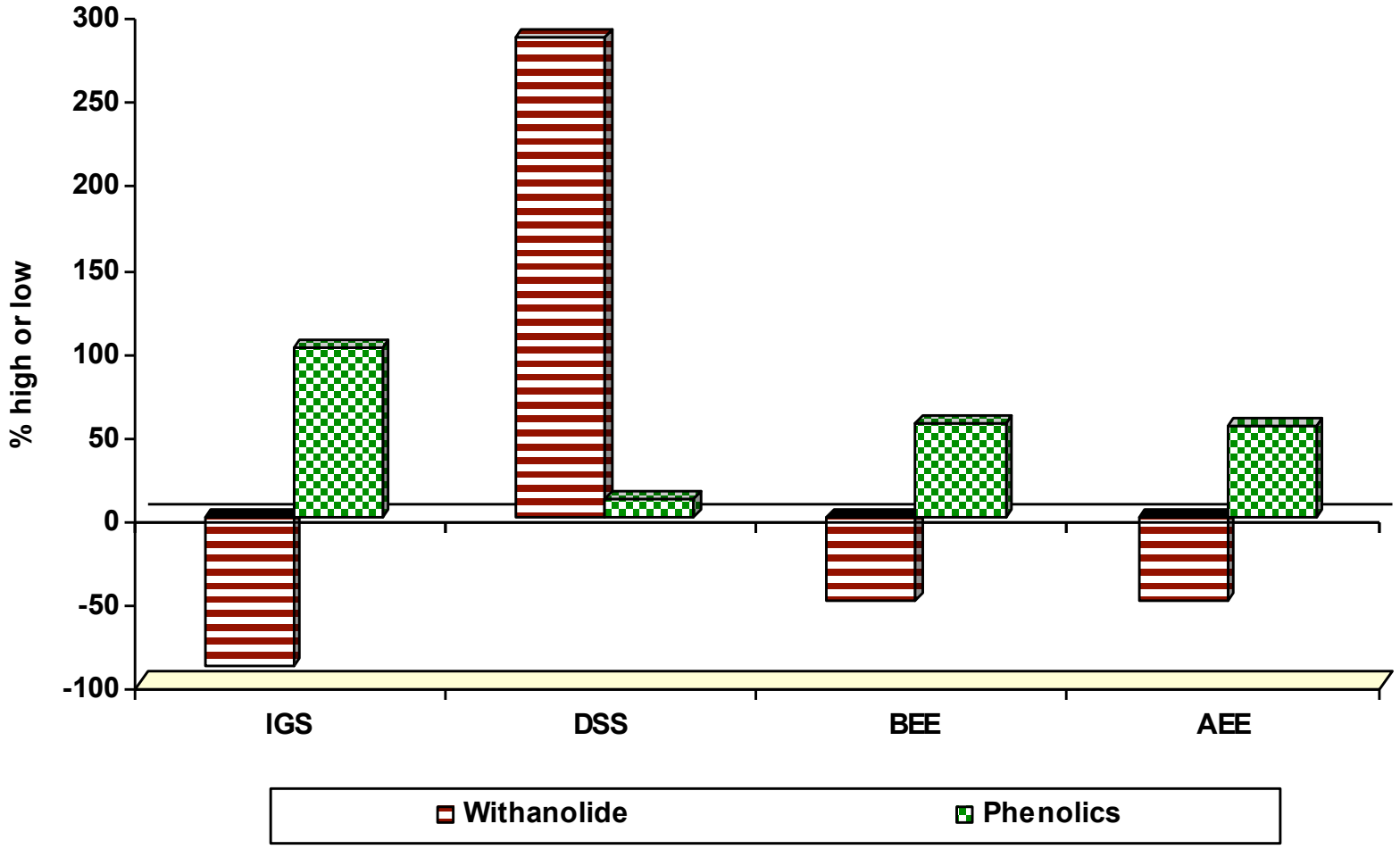


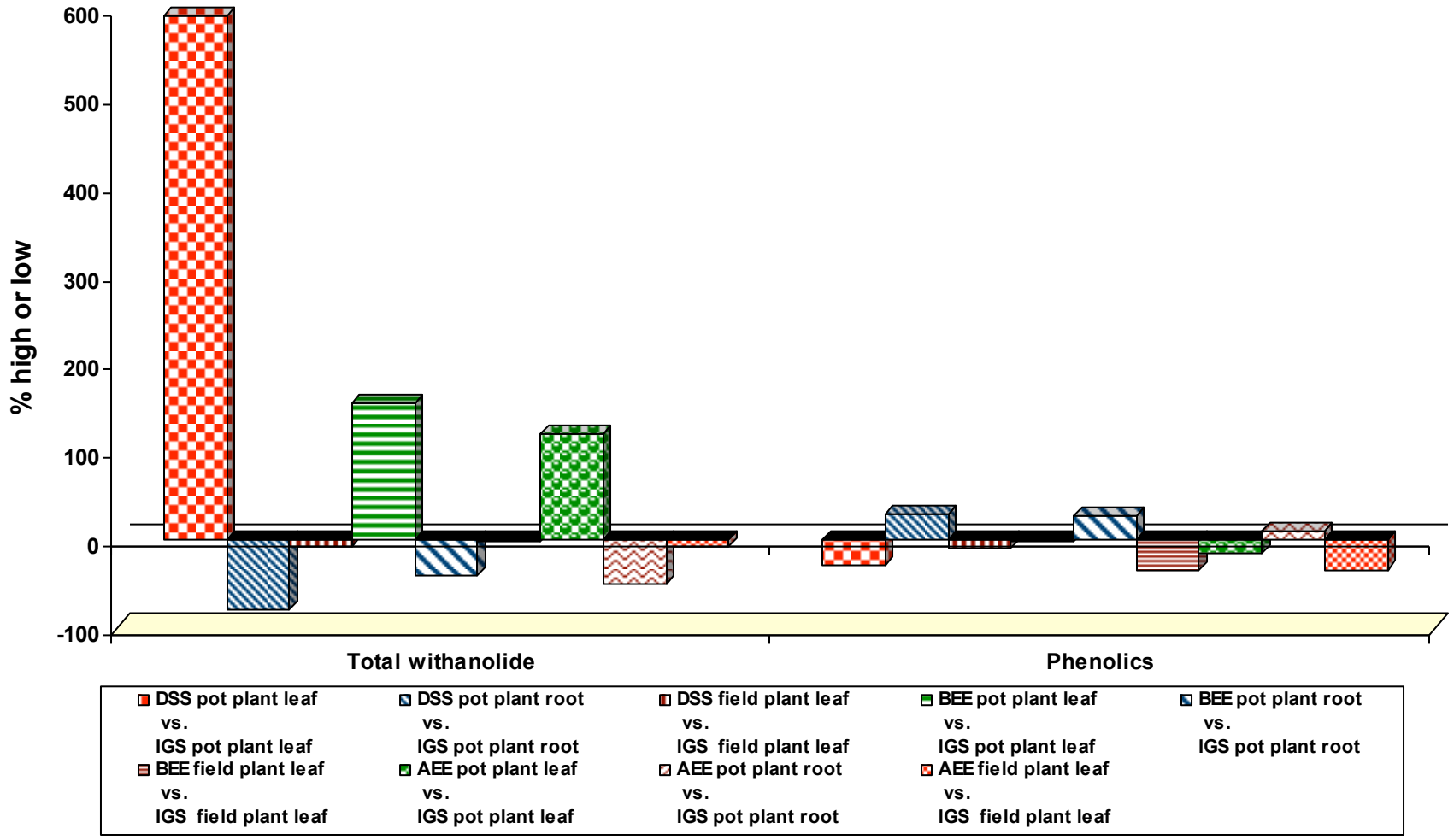
Fig.65 Comparative secondary metabolite status of leaf and roots of 12th month pot plants of *Withania somnifera* grown in selected soil provenances



Withanolides and phenolic content of different plant parts (seeds, leaves and roots) of *Withania somnifera* pots and field grown plants under selected soil provenances

Plant parts	Total no of peaks	Withanolide mg/gdwt	Phenolics mg cinnamic acid eq./gdwt
Seeds	7	0.227	37.18 ± 1.98
Pot plant leaves (IGS)	14	0.015	85.36 ± 8.53
Field plant leaves (IGS)	10	0.069	69.77 ± 9.73
Pot plant roots (IGS)	8	0.132	42.33 ± 12.22
Pot plant leaves (DSS)	11	0.104	60.72 ± 5.18
Field plant leaves (DSS)	12	0.064	63.03 ± 14.36
Pot plant roots (DSS)	9	0.027	54.74 ± 3.94
Pot plant leaves (BEE)	15	0.038	83.52 ± 7.36
Field plant leaves (BEE)	7	0.067	45.33 ± 2.36
Pot plant roots (BEE)	11	0.077	53.54 ± 10.46
Pot plant leaves (AEE)	15	0.033 (2.62×10^{-3})	72.14 ± 3.73
Field plant leaves (AEE)	11	0.063	46.04 ± 3.73
Pot plant roots (AEE)	10	0.066 (6.65×10^{-4})	46.58 ± 7.51

Fig. 87 Comparison of total withanolides and phenolics of leaf and root explants from pot plants and field plants grown on selected soil provenances



MS+1BAP+0.1NAA

Regeneration in

Withania somnifera

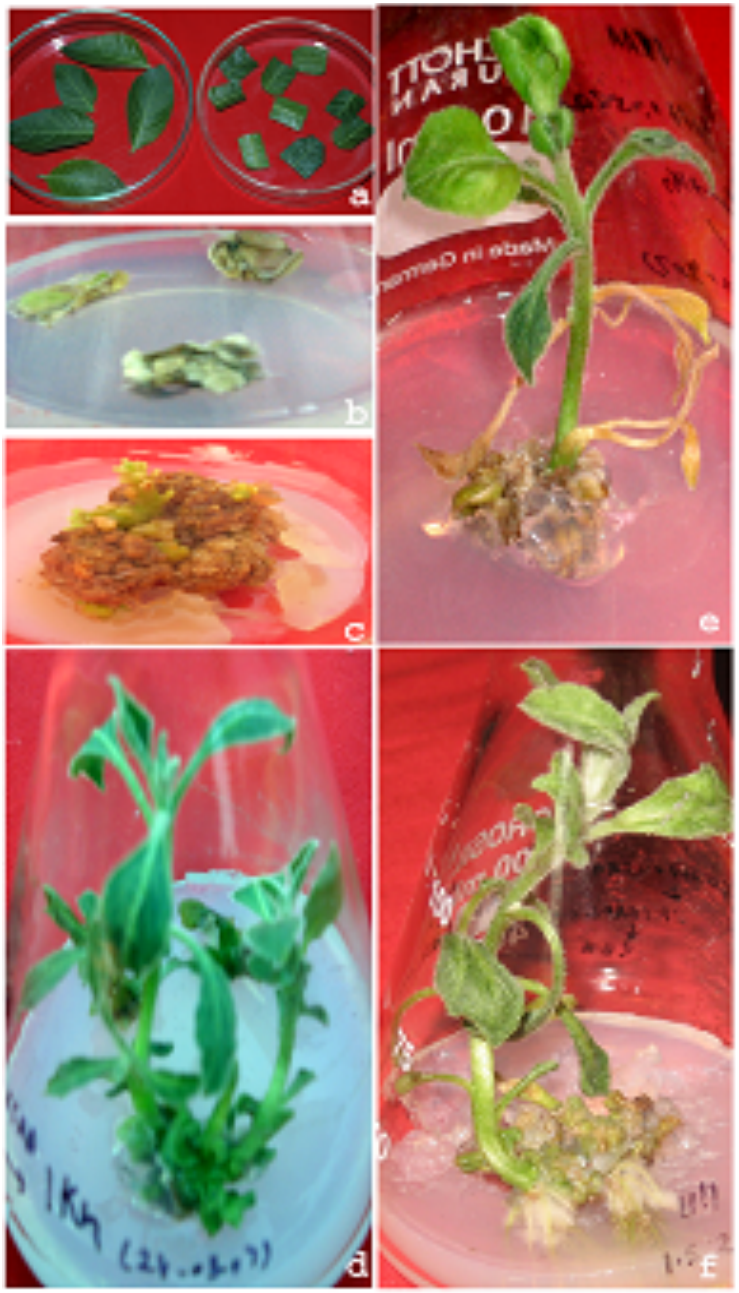
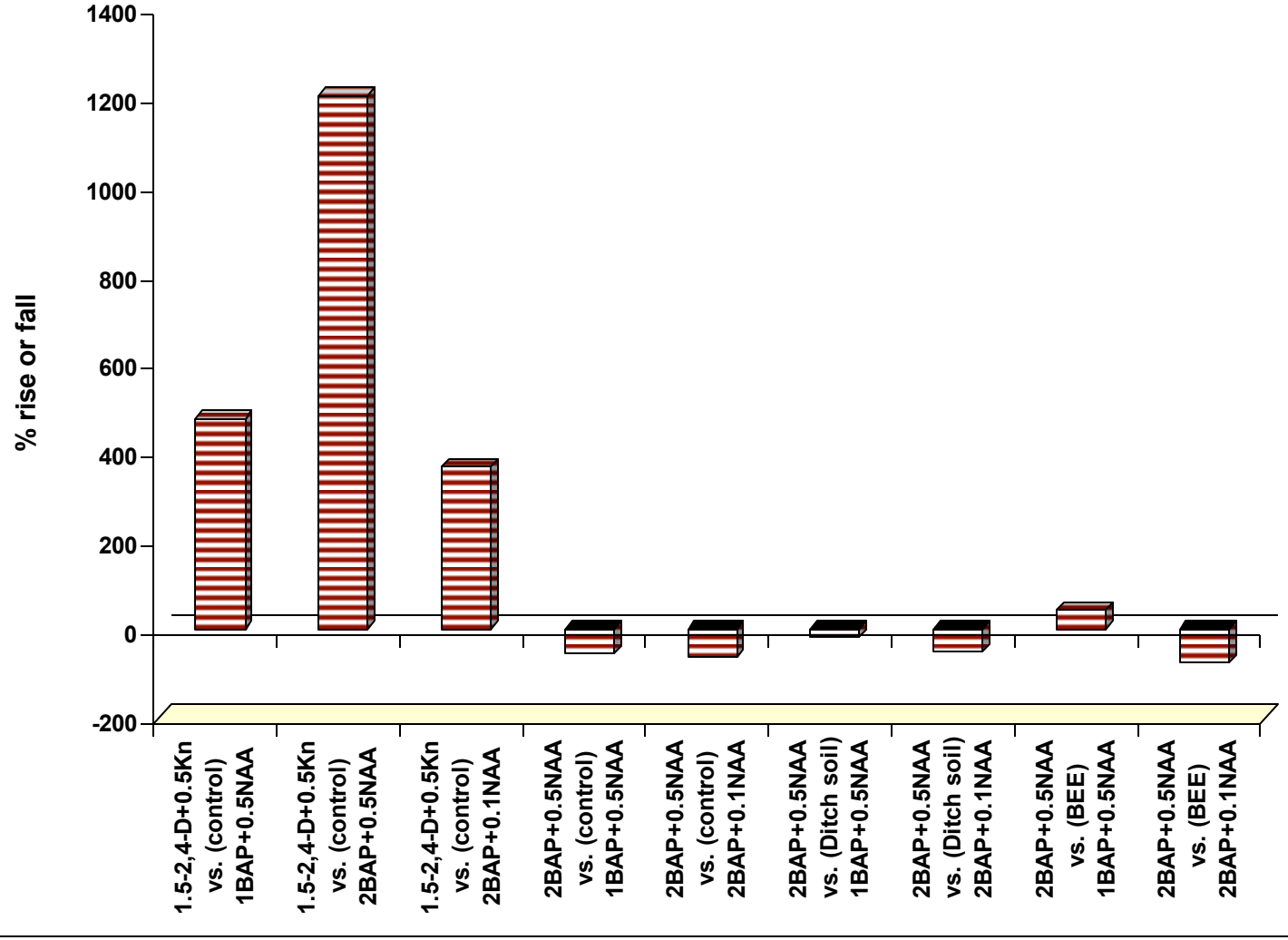


Fig.91 Comparison of calli developed on different media for total withanolides



CONCLUSIONS

5-leaf stage of vetiver plantlets was right stage in terms of tillering, OC and nitrogen status in soil and plant, during 0-5 month study in pots.

Both soil as well as plants underwent rise in %OC in 5-leaf stage plantation during 3^{rd}-4^{th} month in DSS and during 4^{th}-5^{th} month in BEE, indicating C-sequestration whereas in all the other sets % OC either declined in soils or in plants indicating net loss of OC.

Reclamation of all soils in pots or in field, by growing vetiver plants, in terms of accumulation and increased availability of one or more nutrients in the soils.

Rumex spp. on the other hand, growing on all polluted sites, was poor in C:N content, nor did conserve the soil.

Plantlet regeneration from callus developed using AEE grown pot plants was also an achievement.

Callus induced on lower 2,4-D concentration (0.5mg/l) led to accumulation of NRS, phenolics, proteins and nitrogen content exhibiting stressed state, rich in secondary metabolites which could be useful for medicinal purpose.

- On experimental site net gain in OC, nitrogen, phosphorus, K^+ and loss of Na^+ indicates usefulness of *Withania somnifera* in reclamation of soil without its own loss of growth.
- As compared to DSS and BEE leaf (from 12 month-old pot plants), total withanolide content increased in calli, on all the combinations of growth regulators used, and maximum rise being on 2.0mg/l BAP+0.1 mg/l NAA combination, phenolics declined in contrast to withanolides
- The soils exhibited increase in pH. P, K^+ in polluted sites in 12 months.
- OC, Na, K, protein, Reducing sugars increased in potted plants but not in on-site plants. NRS and TS increased in potted and on-site plants.
- In polluted site-grown plants C-N defence compounds accumulated as compared to potted plants, where decline in one component was complemented by increase of the same in soil.

- In both pot and field plants, in general, reclamation of DSS along with considerable growth and yield of required metabolites has been achieved by Vetiver and *Withania* plantation
- AEE soil exhibited accumulation of P and K^+ and decline in Na^+ reducing the possibility of salinity damage to the plants.

UTILITY OF PRESENT INVESTIGATION

Both the selected plants for present study i.e. *Vetiveria zizanioides* and *Withania somnifera* can be used for wasteland reclamation.

Above ground part (leaf) of these plants accumulated more medicinal principles when grown on such sites, and can be used for harvesting the medicinal principles without uprooting them, thus minimizing the pressure on such plants and protecting them from being endangered.

Pharmaceutical companies can utilize such lands for growing medicinal plants, especially *Withania somnifera* and *Vetiveria zizanioides*.

Using explants from plants, growing on such sites, more secondary metabolites can be obtained *in vitro.*


THANK
YOU!