

III - Results And Discussion

1. Germination Studies in C.album :

According to Sen (1977) the germination of seed is one of the most critical phase in the life cycle of the plants as it is subjected to several environmental stresses. Germination is a critical stage which ensures reproduction and consequently controls the dynamics of population, so it is a critical test for the probable crop productivity (Radosevich *et. al* 1997)

a) The effect of NaCl Salinity on germination:

The effect of NaCl salinity on seed germination of *C.album* was studied and the results are shown in table 1.1 From table it is clear that seed germination in *C.album* decreases with increasing NaCl salinity.

According to Huang et.al. (2003) in Haloxylon ammodendron the higher the salinity of NaCl, lower the percentage of germination.

Duan *et.al.* (2004) reported that in *Chenopodium glaucum* germination decreased with increase in salinity. Seed germination percentage was lower in NaCl than in iso-osmotic PEG solutions at osmotic potential less than 0.5 MPa.

Shitole and Dhumal (2008) reported that in *Cassia angustifolia* vehl. seed germination percentage, root and shoot length, seedling height and vigour index, fresh and dry biomass were greatly reduced with increase in concentration of NaCl (25 mM to 100mM) as compared to control.

Sharma *et.al.* (2008) noticed that in *Jatropha curcas.* L. Seed germination reduced with increasing NaCl salinity and at 10EC no seed germination was observed.

Sr. No.	NaCl	Germination %						
	Concentrations	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.		
1	Control	4	80	90	92	98		
2	4 EC	0	28	72	76	90		
3	8 EC	0	14	64	76	88		
4	12 EC	0	4	36	60	72		

 Table 1.1

 Effect of Salt NaCl on Seed germination Calbum

b) The effect of Distillery effluent on seed germination in C.album:

The effect of distillery effluent on the seed germination of *C.album* was studied and the results are shown in table 1.2. It is evident that germination percentage is higher in 40% concentration of distillery effluent as compare to other concentration of distillery effluent and control. Germination is inhibited at higher concentration of distillery effluent (above 60%).

According to Shinde and Trivedy (1983) high concentration of distillery effluent (25 to 50%) inhibited seed germination and reduced seedling height and dry matter production of *Abelmochus exculantus* and *Zea mays* 10 % concentration of distillery effluent is benefited to the seed germination and plant growth. In 1985 Marumkar and Chavan also reported that the germination was completely inhibited by effluent concentration above 20% in *Circer arietinum* L.

According to Pandey et.al (2007) in Wheat (*Triticum aestivum*), Lady's finger (*Abelmochus esculentus*) and pea (*Pisum sativum*) germination percentage decreases with increasing concentration of effluents. In 1987 Jamale reported that in *Phaseolus aureus* germination percentage decreases with increasing concentration of distillery effluent. Karande and Ghanwat (1994) also observed similar result in pigeon pea.

In 1968 Bumbla *et.al.* reported that inhibition of seed germination and growth due to high concentration of effluent. Elecy and Tiwani (1997) reported that in millet crop seed germination was inhibited due to toxic elements present in distillery effluent.

In 1995 Naidu and Raman showed that at higher concentration of distillery effluent germination of peanut was inhibited. Somshekar and Siddaramaih (1998) also reported that in chilli the germination was decreases when treated with heavy metal rich industrial effluent.

Recently some workers also reported that germination of seeds was inhibited at higher concentration of distillery effluent.

Table 1.2

Sr. No.	Distillery	Germination %						
	effluent concentration	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.		
1	Control	4	48	86	86	94		
2	10%	6	70	80	82	86		
3	20%	10	64	70	84	86		
4	40%	4	86	94	96	98		
5	60%	2	76	86	88	88		
6	80%	4	74	82	84	84		
7	100%	4	74	75	76	78		

The effect of Distillery effluent on seed germination in C.album

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	V	A 11	103		percentage	,

c) The effect of Vermiwash on seed germination in C.album:

The results of vermiwash on seed germination of *C.album* is shown in table 1.3. It is clear that seed germination percentage increases with increasing concentration of vermiwash up to 60%, however slightly decreased at higher concentration (e.g. 80% and 100%)

Gopi and Ganeshkumar (2002) reported that the germination percentage of teak seed was found to be higher when treated with vermicast extract as compared to cowdung extract.

Tiwari (1992) reported that alternate wetting and drying with cowdung slurry shows increased germination as compared to alternate wetting and drying with cold water.

According to Vijayanathan (2003) teak seed treated with vermicast extract and vermiwash recorded maximum germination percentage as compared to the other extracts.

Table 1.3

Sr.No.	Vermiwash	Germination %							
	concentration	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.			
1	Control	4	48	60	80	80			
2	10%	6	64	84	92	94			
3	20%	10	46	84	92	94			
4	40%	6	64	80	92	94			
5	60%	2	70	80	86	92			
6	80%	· 0	74	82	88	90			
7	100%	1	52	80	80	82			

The effect of Vermiwash on seed germination in C.album (Values in percentage)

2. Growth Analysis :

He and Cramer (1993) stated that growth analysis is fundamental characterization of a plant responses to an environmental stress. Mainly plant growth can be analysed with respect to the height of plant, root length and shoot length.

a) Effect of NaCl Salinity on plant growth:

The effect of NaCl salinity on plant growth and development has been recorded in the table 2.1. From the table it is clear that at 8 E.C. NaCl treatment plant shows higher growth than the control plant. However it is decreased at higher NaCl salinity. In 1971 Muhammad and Muhammad reported that at higher salinity reduction in height of sunflower plant was due to reduced physiological availability of water during the vegetative growth period.

Karadge and Chavan (1977, 1981) observed an adverse effect of salinity stress on height of groundnut CV. SB-11 plant. Effect of soil salinity on different species of *Amaranthus* was studied by Gaikwad (1995). He observed that height of Amaranthus caudatus was increased sharply with the increasing concentration of salinity (NaCl) in rooting medium; but height of *A. hypochondricas* and *A. paniculatus* were increased at lower and moderate level of salinity.

According to Mohammad (2002) the height of paper hybrid plant was found stimulated by lower levels of salinity but it was significantly reduced with increasing concentration of NaCl in the growth medium. Kumar *et. al.* (2005) reported that the height of *Brassica juncea* was decreased with increasing salinity.

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Effect of Salinity (NaCl) on plant growth in C.album

Sr.No.	NaCl concentration	Height in cm.
1	Control	17.6
2	4 Ec	15.3
3	8 EC	18.1
4	12 EC	15.3

b) Effect of Distillery effluent on plant growth:

The results of the effect of different concentrations of distillery effluent on C. *album* are depicted in table 2.2. The results show that height of C. *album* is the highest at 40% and it is decreased with increasing concentration of distillery effluent. It is also observed that plants can not survive and die off within a week after the treatment with concentrated (100%) distillery effluent.

Cannabiran and Prasad (1997) reported better performance of *Vinja mungo* plant in diluted distillery effluent application. In 1995 Naidu and Raman reported that growth decreases with increasing concentration of distillery effluent. Spent wash contains NPK and hence it is used as the source of manure of potassic fertilizer or as an irrigation water for agronomic purpose of soil Patil *et. al.* (1995). Utilization of organic matter and micronutrient rich fermentation effluents after suitable dilution as liquid fertilizer was recommended by Swaminathan *et. al.* (1992),Om Hari *et.al* (1994) and Pandey and Sohni (1994).

Shinde and Trivedy (1983) reported that height of *Abelmoschus esculantus* was increased in 10% and 25% but it was less in 50% distillery effluent treatment. In case of *Zea mays* growth increases by using distillery effluent. However slight reduction in the height of seedlings as the concentration increases. In 1999 Pathak *et. al.* noted that soil amendment with diluted fermentation effluent increased yield of wheat and rice.

Table 2.2

Sr.No.	Distillery effluent concentration	Height in cm.
1	Control	13.6
2	10%	10.6
3	20%	10.3
4	40%	12.6
5	60%	8.9
6	80%	8.6
7	100%	No Survival of plants

Effect of Distillery effluent on growth in C.album

c) Effect of vermiwash on plant growth:

The effect of vermiwash spray on growth and development with respect to plant height of *C. album* are recorded in Table No. 2.3. It is clear that average height of *C.album* increases at 10% concentration of vermiwash as compare to other concentration.

Gopi and Ganeshkumar (2002) studied the effect of vermicompost application on teak seedlings at different ratios and reported that vermicompost produce maximum mean shoot length and also produce maximum number of leaves per plant. In 2004 Sivasubramanian and Ganeshkumar observed the effect of vermiwash on African marigold. The results revealed that vermiwash spray enhanced the plant growth parameters that is plant height, number of laterals, number of leaves and leaf area. Vijaya, Shivthramma and Swamy (2006) reported that growth of *Cyamposis tetragonoloba* plants were increased by the application of vermicompost over control.

In 2008, Dasa reported that due to application of vermiwash spray on sugarcane plants, the yield increases upto 65-75 tonnes from 55-60 tonnes per acre.

Table 2.3

Sr.No.	Vermiwash concentration	Height in cm.	
1	Control	7.9	
2	10%	10.8	
3	20%	10.2	
4	40%	10	
5	60%	9.9	
6	80%	9.6	
7	100%	9.5	

Effect of vermiwash on growth in C.album

3. Photosynthetic Pigments:

A) Chlorophylls:

Chlorophylls are green coloured pigment that play very important role in photosynthesis. Due to drought, salt stress and senescence chlorophyll content decreases. By application of fertilizers like vermiwash, distillery effluent chlorophylls increase upto certain level.

a) The effect of NaCl salinity on Chlorophylls:

The results of the effect of NaCl salinity on Chlorophyll contents of *C. album* are depicted in table 3.1. It is clear from the results that total chlorophylls are higher in 4 EC NaCl treatment than control. Chlorophyll- a, chlorophyll - b are also higher in 4 EC treatment than control but it decreases with increasing salinity .Chlorophyll a:b ratio is also decreasing with increasing (salinity) concentrations of NaCl.

Karadge and Chavan (1981) reported that chlorophyll content in *Archis hypogea* was decreased with increasing salinity. Strogonov (1973) reported that chlorophyll and carotenoid contents increase in the salt sensitive plant due to salt stress. In 1995 Mishra *et. al.* observed that Chlorophyll content of *Brassica juncea* was increased at lower concentration of NaCl salinity but it was decreased with increasing salinity. Gababuer *et.al.* (2003) also reported the similar types of observation in *Adansonia digitata*.

Chlorophyll contents decreased with increasing salinity was reported by number of researchers (Murumkar and Chavan (1986) in Chickpea), Misra *et.al.* (2006) reported similar results in Kidney bean seedling; Karadge and Chavan (1981) in groundnut; Bhivare and Nimbalkar (1984) in French beans reported that chlorophyll contents decreased with increased NaCl salinity but it was increased with Na₂So₄.

Shimose (1972) in *Asparagus* and Siegel and Bjarsch (1962) in tomato, Strogonov *et.al.*(1970) in Cabbage reported that chlorophyll decreased with increased salt stress.

In 1992 Morales *et.al.* observed that salinity induced significant changes in chlorophyll fluroscence induction curve from Barley leaves. Gaikwad (1995) noticed that in *Amaranthus caudatus* and *A. hypochondriacus* chlorophylls content decreased with increasing salt stress but it was increased at lower and moderate salt levels in *A. paniculatus*.

Lycoskoutis *et.al.* (2005) reported that net photosynthesis and chlorophyll content in *Capscium annum* decreased due to salinity treatment.

Table 3.1

Sr. No.	NaCl concentration	Total Chl-a	Total Chl-b	Chl a / b Ratio	Total Chlorophylls a+b	Carotenoids
1	Control	58.3	41.61	1.4	99.88	12.64
2	4 EC	64.73	47.78	1.35	112.48	13.96
3	8 EC	57.55	50	1.15	107.51	14.56
4	12 EC	43.15	32.96	1.30	76.08	10.4

Effect of salt (NaCl) on photosynthetic pigments in *C. album* Values expressed in mg/100g fresh leaf tissue





b) The effect of Distillery effluent on Chlorophylls:

The effect of different concentrations of distillery effluent on chlorophyll content of leaves of C. *album* is shown in table 3.2. From the result it is concluded that chlorophyll content is increased upto 40% distillery effluent but it is decreasing with increasing concentration of distillery effluent. Chlorophyll a, b and total

chlorophylls in C. album at 40% concentration of distillery effluent is higher than control.

According Verma and Verma (1995) chlorophyll content was increased with increasing concentration of spent wash treatment in *Lens culinaris* L. Datta and Biossya (1998) observed the same results in *Oryza sativa* L. var masurin. In 2000 Bhosale reported in sugarcane chlorophylls increased with increasing concentration of spentwash.

Behera and Misra (1981) reported that in rice seedlings chlorophyll content was decline with increase in effluent concentration and the time of incubation. In 1991 Behera and Misra also reported that chlorophyll contents in rice seedling were decreasing with increase in effluent concentration and time. Raman (1995) observed that chlorophyll content decreased with increasing concentration of spent wash in ground nuts.

Sharma *et.al.* (2002) studied the effect of distillery effluent on pigment content of sugar beet. They observed that higher concentration i.e. above 5% of effluent were found to be toxic, however it can be used for irrigation purpose after proper dilution.

Roy *et.al.* (2008) reported that in rice the content of chlorophyll-a and chlorophyll-b were increase with increase in concentration of effluent. They also reported that increased effluent concentration may denature the chlorophyll protein which subsequently affect the photosynthetic apparatus.

Decreasing the chlorophyll content altimately decrease the plant productivity. Pandey and Sohni (1994) reported higher Chlorophyll content in the forest seed beds treated with lower concentration of distillery effluent. According to Siddaramaih *et.al* (1998) the germination and chlorophyll in chilli has been degraded when treated with heavy metal rich industrial effluent.

Table 3.2

Sr. No.	Distillery effluent concentration	Total Chl-a	Total Chl-b	Chl a / b Ratio	Total Chlorophylls a+b	Carotenoids
1	Control	49.42	34.36	1.43	83.76	10
2	10%	34.52	26.71	1.29	61.22	8.2
3	20%	36.8	28	1.31	64.8	8.4
4	40%	51.42	38	1.35	89.4	12
5	60%	39.3	27	1.45	66	8.8
6	80%	39	29	1.32	68	9.2

Effect of distillery effluent on in *C. album* photosynthetic pigments Values expressed in mg/100g fresh leaf tissue

Photosynthetic pigments



c) The effect of Vermiwash treatment on Chlorophylls:

The effect of vermiwash spray on the chlorophyll content of *C. album* shown in table 3.3. From results it is evident that chlorophyll content Chl-a and Chl-b are more in 10% concentration of vermiwash spray as compared to other concentration and control.

Gopi and Ganeshkumar (2002) reported that among the other treatment the teak seedling treated with vermicompost showed the highest chlorophyll content.

Vijayananthan (2003) reported the application of vermicompost at different ratios significantly increated the growth and biomass production at teak.

According to Sindhumol (2008) if 10% vermiwash sprayed on the plants it showed the best growth of plants. In the present investigation *C.album* also shows higher chlorophyll content in 10% vermiwash spray.

According to Vijaya et.al.(2006) growth of Cyamposis tetragonoloba was increased by vermicompost application.

Sivasubramanian and Ganeshkumar (2004) reported that the maximum mean number of leaves per marigold plant were produced due to foliar application of vermiwash.

Hence vermiwash increases growth and yield of plants i.e. it is the best tonic for the plants.

Table 3.3

Effect of vermiwash on photosynthetic pigments in *C. album* Values expressed in mg/100g fresh leaf tissue

Sr. No.	Vermiwash concentration	Total Chl-a	Total Chl-b	Chl a / b Ratio	Total Chlorophylls a+b	Carotenoids
1	Control	75.4	52.2	1.44	127.5	16.16
2	10%	95.2	72.1	1.32	167.2	21.12
3	20%	75.50	51.26	1.47	126.73	14.76
4	40%	65.29	47.14	1.38	112.40	14.72
5	60%	64.84	42.70	1.51	107.4	12.72
6	80%	54.23	46.18	1.17	100.38	12.4
7	100%	43.63	40.97	1.06	97.28	12.12



d) The effect of Senescence on chlorophylls:

Effect of leaf senescence on the chlorophyll content of *C.album* is shown in table 3.4. From fig. it is concluded that chlorophyll content decreased as leaf becomes senescent. The total chlorophyll content is higher in premature leaves of *C. album* as compare to young mature and progressively older leaves and very much reduced amount of total chlorophyll, (Chl -a, b and total Chlorophylls) are present in senescent leaves.

Fletcher and Osborne (1965) and Fletcher (1969) reported that during senescence there was decline in chlorophyll content, proteins and nucleic acids.

According to Salunkhe (1997) chlorophyll contents in senescent leaves of Ipomoea cornea were decreased.

Lamattina *et. al.* (1987) reported that in cotton leaves Chl-a and Chl-b contents were declined with leaf senescence. Dyer and Osborne (1971) reported that leaves of plant approaching to senescence the synthesis of nucleic acid and protein becomes progressively less and total levels fall. Quantitative changes in the pigment composition are also found in senescing leaves.

Joshi (1988) reported that there was increase in Chl-a and Chl-b in mature leaves and markedly decrease in senescent leaves of custard apple, mango and guava.

Table 3.4

Effect of senescence on photosynthetic pigments in *C. album* Values expressed in mg/100g fresh leaf tissue

Sr.No.	stages of leaf	Total Chl-a	Total Chl-b	Chl a / b Ratio	Total Chlorophylls a+b	Carotenoids
1	Young	89.34	61.72	1.44	151.00	19.6
2	Premature	116.16	71.33	1.62	187.45	21.44
3	Mature	102.50	71.8	1.4	173.2	19.48
4	Onset of senescence	27.40	23.20	1.18	50.59	7.36
5	Senescent	5.147	6.87	0.75	12	6.24



Photosynthetic pigments

B) Carotenoids :

Carotenoids are lipid soluble pigments found in all plants. They are synthesized and located in the chloroplasts along with chlorophylls. Carotenoids acts as accessory pigments in photosynthesis that is they absorb light energy and transfer it to chlorophylls. They also protect the chloroplast from photooxidation damage.

a) The effect of Salinity (NaCl) on Carotenoids :

The effect of NaCl salinity on carotenoids of *C. album* is shown in Table 3.1. From the results it is clear that carotenoids are higher in 8 EC than control but it decreases with increasing NaCl salinity.

According to Hamada (1996) the carotenoid contents were also increased in wheat when subjected to either salinity or drought or both. Hamada and Enany (1994) reported that the concentration of carotenoids were increased in broad bean leaves while they remained more or less unaffected up to 80 mM NaCl and there above decline significantly in pea plants.

According to Hebbar (2008) salinity reduced both leaf area expansion and photosynthesis in all cultivated species of cotton (*Gossypium* spp.). Some workers reported that reduction in carotenoid contents is due to salinity stress. (Ali *et.al.* 1992) in *Brassica juncea*; Agastion *et.al* (2000) in Mulberry, and Parida *et.al* 2005 in *Aegiceros corniculatum*.

Reduction in crotenoids due to salinity stress has been reported by Rao and Rao (1981) in *Cajanus indicus* and *Sesamum indicum* plants but this reduction was more pronounced in *Sesamum indicum*.

According to Reddy and Vora (1986, 1987) in *Pennisetum typhoidus* and wheat leaves carotenoids declined with increasing salinity. Our results are in the similar lines.

b) The effect of Distillery effluent on Carotenoids:

The effect of distillery effluent on carotenoids in *C. album* is shown in table 3.2. Our results show that carotenoid contents are higher in 40% concentration of distillery effluent than other concentrations of distillery effluent and control.

According to Singh (1982) the inhibition of photosynthetic activity in leguminous plants and reduction in chlorophyll content may cause osmotic effect of high effluent concentration which reduce the uptake of Mg^{2+} , k^+ and mineral ions thereby inhibiting the synthesis of pigment and affecting the photosynthesis. Further he stated that decrease in carotenoids may be either because of its degeneration due to toxic elements present in distillery effluent.

Bhosale (2000) reported in sugarcane that carotenoids level goes down if the effluent concentration exceeds Tb 1000 and no major change in the carotenoids level with increasing concentration. of effluent was observed in 12 month's age crop except at Tb 500 and Tb 1000.

In 2002 Sharma *et.al.* reported that there was toxic effect of distillery effluent treatment on pigment content of sugar beet.

Beheral and Misra (1981) reported that the total pigments of rice seedlings declined with an increase in effluent concentration and time of incubation.

Pragasam and Kannabiran (2001) observed the effect of distillery effluent on the growth, biochemistry and yield of <u>Vinga mungo</u> and concluded that all parameters increase over control in 10% and 20% concentration. At higher concentration (above 50%)growth and yield were reduced.

c) The effect of Vermiwash on Carotenoids:

The effect of vermiwash on carotenoid content in *C. album* is shown in Table 3.3. From the results it is clear that carotenoids are higher in 10% Vermiwash foliar treatment than other concentrations of vermiwash and control.

R. Thriravenkataswamy concluded that vermiwash applied as 10% foliar spray that acts as a growth promoter.

Sivasubrumaniam and Ganesh-Kumar (2004) observed that vermiwash spray enhanced the growth parameters that is plant height, number of laterals, number of leaves and leaf area. In marigold plant productivity is also enhanced by vermiwash spray application.

Gajalakshmi *et.al.* (2002) observed that application of vermicompost leads to significant improvement in growth and flowering as compared to the untreated plants.

d) The effect of Senescence on carotenoids :

The effect of leaf aging or senescence on carotenoids in *C. album* is shown in table 3.4. From our results it is clear that premature leaves of *C. album* contain high carotenoids as compare to young, mature, onset of senescence and senescent leaves. Senescent leaves contain very less amount of carotenoids.

Rane (1991) reported that carotenoids decreased during leaf senescence was comparatively significant in groundnut leaves during induced leaf senescence.

Choe and Thiamann (1975) observed that rapid loss of both chlorophylls and carotenoids during aging of isolated chloroplast in light. Panda and Biswal (1989) found that both the chlorophylls and carotenoids are degraded but carotenoids are degraded faster than chlorophylls. However in case of detached barley leaves Biswal and Mohanthy (1976) found that chlorophyll are degraded much faster than Carotenoids.

4. Total polyphenols:

Polyphenols are aromatic compounds formed during secondary metabolism in plants. Most of these are phenolics intermediates and derivatives of the shikimate and phenylpropanoid pathways (Cheng and Breen, 1991). The Phenolic compounds affect the growth and other activities of plants like photosynthesis, chlorophyll synthesis, protein synthesis, water relation and respiration.

a) The effect of NaCl salinity on polyphenols:

The results of the effect of NaCl salinity on polyphenol contents of C. *album*. are shown in Table 4.1. From the results, it is clear that polyphenol increases with increasing salinity stress.

In 1964 Strogonov reported that salinity stimulates secondary metabolism in plants and polyphenol contents increases. Number of workers pointed out that polyphenol content increases with increasing NaCl salinity (Karadge and Chavan 1981 in groundnut variety T.M.V. 10; Wadkar (1989) in *Crotolaria retusa*; Parida *et.al.* (2005) in *Aegiceros corniculatum*; Singh and Kumari (2006) in *Brassica compastris*.

Joshi (1970) pointed out that highly evolved halophytes mangroves are rich in polyphenols. In 1988 Nalawade reported that polyphenol contents increases with high salinity stress in *Nigar*.

Krishnamoorthy and Siddique (1985) in Cowpea reported that decrease in level of phenolic compounds under saline conditions. The total alkaloid content of Datura was increased due to salinity (Brachet and Cosson, 1986).

Table 4.1

Sr.No.	NaCl concentration	Total Polyphenols
1	Control	1.750
2	4 EC	2.357
3	8 EC	2.750
4	12 EC	3.000

Effect of NaCl on total polyphenol contents in *C. album* Values expressed in g/100g fresh leaf tissue



b) The effect of Distillery effluent on polyphenols:

The results of effect of distillery effluent on polyphenol content of *C. album* are shown in table 4.2 and it is evident that polyphenol contents increase with increasing concentration of distillery effluent. The highest polyphenols are observed at 60% and 80% distillery effluent treatment.

Kannan and Upreti (2008) treated mung bean with distillery effluent and observed that the leaching of carbohydrates and proteins were much higher in case of untreated effluent than treated plants with effluent.

In 2008 Ranu Roy et.al. reported that in rice plant increased effluent concentration may denature the chloroplast, protein which subsequently affect the

photoshynthetic apparatus decreasing the chlorophyll content with concomitant decrease in plant productivity.

Pragasum and Kannabiran (2001) studied the effect of distillery effluent on *Vigna mungo* and concluded that all parameters showed an increase over control. In 10 and 50% concentration but at higher concentration (above 50%) were found to reduce growth and yield.

Table 4.2

Effect of distillery effluent on total polyphenol contents in *C. album* Values expressed in g/100g fresh leaf tissue

Sr.No.	Distillery effluent concentration	Total Polyphenols
1	Control	1.45
2	10%	1.714
3	20%	1.678
4	40%	1.535
5	60%	2.142
6	80%	2.107



c) The effect of vermiwash on polyphenols:

The results of the effect of vermiwash on polyphenol content in *C. album* are shown in table 4.3, from results it is clear that polyphenol contents increases with increasing concentration of vermiwash. The highest polyphenols are recorded at 20% and 10% concentration of vermiwash.

Table 4.3

Effect of vermiwash on total polyphenol contents in C. album

Sr.No.	vermiwash concentration	Total Polyphenols
1	Control	2.021
2	10%	2.235
3	20%	2.220
4	40%	1.878
5	60%	1.678
6	80%	1.564
7	100%	0.821

Values expressed in g/100g fresh leaf tissue



d) The effect of Senescence on polyphenols:

The result of the effect of senescence on the polyphenol contents in the *C*. *album* is shown in table 4.4. The highest polyphenol contents are recorded in premature leaves. Further polyphenol content decreases from mature to senescent stage of leaf.

Bhatia *et. al.* (1971) have reported that high tannin contents in young leaves of *Zggium cumuni* while it decreased at maturity. Kar and Mishra (1976) reported that total phenolics accumulated in detached and darkened rice leaves, but in attached leaf senescence in light no accumulation of phenolics was observed.

According to Joshi (1988) in Pomegranat and Guava the mature leaves contain relatively higher level of total polyphenols and in senescent leave total polyphenols were decline slightly.

Shetty (1971) found that young leaves of *Acrosticum aureum* were low in phenols. Whereas the phenols reached their maximum value in mature leaves and old leaves show decreased in phenol contents. In 1975 Jamale reported that polyphenols were decreased in senescent leaves of mangrove species e.g. *Sonneratia acida* and *Lumnitzera racemosa*.

Table 4.4

Effect of leaf senescence on total polyphenol contents in C. album.

Sr.No.	Leaf stage	Total Polyphenols
1	Young	4.928
2	Premature	6.678
3	Mature	4.214
4	Onset of senescence	3.821
5	Senescent	1.750

Values expressed in g/100g fresh leaf tissue



5. Carbohydrates:

Carbohydrates are the most important components because they are potential source of energy which is essential for biochemical reactions of cells. Carbohydrates mainly divided into 3 groups monosaccharides, oligosaccharides and polysaccharides. According to Morgan (1992) beside the key role in carbon metabolism the energetic of plant cell, carbohydrates particularly soluble sugars play an important role in osmoregulation.

a) The effect of NaCl Salinity on Carbohydrates:

The results of the effect of NaCl salinity on carbohydrates of *C. album* are shown in table 5.1, from table it is evident that total sugars, reducing sugars and starch increases with NaCl salinity. At 8EC treatment total sugars, reducing sugars and starch are higher as compared to others. But carbohydrates are markedly decline at 12 EC NaCl treatment.

According to Agustian *et.al* (2000) the content of soluble sugars sucrose and starch in mulberry genotypes were increased at lower salinity level and then decreased at higher concentration of salt.

According to Rathert *et.al.* (1981) the level of reducing sugars in the leaves of salt tolerant sugarbeet decreased due to salt treatment.

In 1999 Gaikwad and Chavan noticed that the non reducing sugars, reducing sugars and starch contents in all parts of *Amaranthus* species were increased at lower NaCl salinity but decreased at higher salinity stress. Matar *et.al.* (1975) noticed that in salt sensitive lettuce sucrose contents decreased in the leaves and root considerably due to salinity.

Ninknam *et.al.* (2004) pointed out that the concentration of reducing sugars Oligosaccharides, soluble sugars and total sugars in the seedlings of *Nicotiana tobaccum* decreased with increasing levels of salinity upto 150 mM. NaCl and then increased at higher concentration of NaCl.

Chavan (1980) reported a decline in carbohydrates in ragi grains when the plants were subjected to NaCl in sand culture experiment.

Doering and Luedders (1986) also noticed in pomegranate that in all salt variations the amount of reducing sugars in the leaves was increased whereas the roots showed a reduced concentration of the fraction.

Huber (1989) noticed that leaves of broad bean, pea and spinach accumulated more sucrose than starch Kabuzenko and Ponomerova (1980) reported that in salt resistance tomato starch content was considerably increased with increasing salinity.

In 2003 Murphy *et.al.* reported that total carbohydrates in *Ruppia maritima* was decreased by 65% while the soluble sugar was increased by 34% and which might acts as compatible solute at higher levels of salinity.

Table 5.1

Effect of NaCl on Carbohydrate contents in *C. album* Values expressed in g/100g dry matter

Sr. No.	NaCl concentration	Reducing Sugars	Total Sugars	Starch
1	Control	0.460	1.50	15.77
2	4 EC	0.314	1.80	9.90
3	8 EC	0.944	2.03	20.74
4	12 EC	0.510	1.60	11.1

b) The effect of Distillery effluent on carbohydrates:

The results of the effect of distillery effluent on carbohydrate contents in C. *album* is shown in table 5.2, from the results it is clear that reducing sugars, total sugars and starch contents in C. *album* leaves were increased up to 10 to 20% distillery effluent treatment and decreased from 40% concentration.

According to Kumar (2001) in *Hordeum vulgare* var. IB65 after treatment of distillery effluent there was reduction in carbohydrates and crude proteins. Hence he stated that sugar - mill effluent produces adverse effect on soil fertility and chemical constituents of plant. if used for irrigation purposes.

Pragasum and Kannabiran (2001) observed that the effect of distillery effluent on *Vigna mungo* and reported that all the parameters are increased over control and in 10% to 20% concentration but at higher concentration there was reduction in growth and yield.

According to Sharma *et.al.* higher concentration of effluent were found to be toxic, however it can be used for irrigational purpose after proper dilution. In 1991 Misra and Behera stated that Carbohydrate contents decreased in rice seedlings with increasing concentration of effluent and time.

The yield of sugarcane increased by 64% to 71% when fertilized with properly diluted distillery effluent alone and by 58% to 61% in combination with area sugar yield increased by as much as 93-95% over the unfertilized plants (News and Events may 2, 2008).

Table 5.2

Effect of distillery effluent on Carbohydrate contents in C. album Values expressed in g/100g dry matter

Sr.No.	Distillery effluent concentration	Reducing Sugars	Total Sugars	Starch
1	Control	0.65	1.86	17.65
2	10%	1.6	2.53	20.12
3	20%	1.0	1.79	22.41
4	40%	0.86	1.61	18.10
5	60%	0.63	1.32	16.22
6	80%	0.70	1.42	16.85

c) The effect of Senescence on carbohydrates:

The results of the effect of senescence or leaf aging on carbohydrate contents in *C. album* are shown in table 5.3, from results it evident that reducing sugars and total sugars are high in premature leaves and they become less in senescent leaves. Starch content is high in young and mature leaves however it decreased in senescent leaves of *C. album* due to good translocation.

According to Lewington and Simon (1969) carbohydrates were declined in senescent leaves. Baur *et.al.* (1968) have noted that, aged leaves of tobacco hydrolysed carbohydrate gets translocated to the other parts of the plant. According Yakimoto *et. al.*(1978) reducing sugars and total sugars were declined in older leaves while starch content was increased.

Roadof and Hofner (1971) noticed that in older leaves of *Phaseolus* incorporation of C^{14} in sugar fraction was more pronounced. Similar trend was recorded in older leaves of cotton (Chnb,1975).

Joshi (1988) reported that in custard apple reducing sugars were higher in young and mature leaves and they were declined in senescent leaves. Total sugars were at the highest level in mature leaves and decreased in senescent leaves. They also showed that starch was more in all stages of leaf.

Table 5.3

	· mues expressed in 5, roog ury matter							
Sr.No.	Leaf Stage	Reducing Sugars	Total Sugars	Starch				
1	Young	2.54	3.26	16.77				
2	Premature	3.77	6.31	20.12				
3	Mature	2.51	4.92	24.20				
4	Onset of senescence	2.34	4.30	29.14				
5	Senescent	1.85	3.77	19.80				

Values expressed in g/100g dry matter

Effect of leaf senescence on Carbohydrate contents in C. album

6. The effect of Senescence on Oxalic acid:

In *C.album* oxalic acid is the major organic acid found besides the conventional acids of TCA cycle in number of plants. It is a dicarboxylic organic acid present in soluble and insoluble forms. The soluble forms consist mainly of K-oxalate and Mg-oxalate and insoluble form of Ca-oxalate (Vityakon and Standal, 1989). According to Waisel (1972) the high oxidation level of oxalic acid and its accumulation to high levels in leaves, it is involved in energy yielding metabolic processes and in generation of active formally groups.

In the present investigation oxalic acid content was studied in young to senescent leaves of *C.album*. The results are depicted in table 6.1 It has been found that oxalate contents increased in mature and premature leaves of *C.album*. However it decreases in senescing and senescent leaves.

According to Singh and Saxena (1972) noticed that oxalic acid content of leafy vegetables *A. gangetices, chenonodium murale, C.album. portulaca oleracea* and *Beta valgaris* were uniformly distributed in the leaves and stem during early stages of growth while in latter stage of maturity they generally increased in the leaves and decreased in the stem. In 1972 Waisel reported that among halophytes oxalic acid is the major organic acid.

Osmond (1966) found that extensive absorption of calcium from calcium chloride solution by *Atriplex* spp. was not accompanied by net synthesis of oxalate.

According Osmond (1967) in *A. inflata* and *A. vesicaria* total oxalate level declined as calcium absorption increased.

Osmond (1963), Albert and Popp (1977) reported that the high levels of oxalic acid were observed in some halophytes of chenopodiaceae.

Karadge (1981) studied *Portulaca oleracea* and noticed that there was continuous drop in K^+ as well as Ca⁺⁺ contents in the salt treated leaves that may affect the total oxalate content and specifically bound oxalic acid.

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Sr.No.	Leaf stage	Oxalic Acid %
1	Young	0.0786
2	Premature	0.1431
3	Mature	0.1890
4	Onset of senescence	0.1861
5	Senescent	0.176

Effect	of	senescence	on	oxalic	acid	in	С.	album
	•••						~.	



7. Inorganic Constituents:

Different types of macro and micro elements are required for plant growth and development. In favourable conditions plant can absorb essential elements in sufficient quantity but adverse conditions affect mineral uptake, transport, distribution through out the plant body. In the present investigation the effect of different treatment on mineral uptake in *C.album* has been undertaken.

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A) Sodium:

Sodium is an important macronutrient because it stimulate the growth by cell expansion and water balance of plants. Role of sodium in maintaining favourable water balance has been suggested by Rains (1972). According Evans and Sorger (1966) sodium acts as activater for some enzyme systems. Kylin (1973) reported that stimulation of ATPase takes place in presence of Na. According to Rozema (1991) and Glenn *et.al.* (1992) the high sodium physiotype is associated with the most salt tolerent species of dicotyleden and euhalophytes typified by the species in the chenopodiaceae.

a) The effect of NaCl Salinity on Na in C. album:

The results of NaCl salinity on sodium concentration of *C.album* are shown in table 7.1. It is clear that sodium concentration increased with increasing NaCl salinity.

According to Osmond *et.al.* (1980) accumulation of Na in chenopodiaceae members is well documented and they have been termed craving for salt. Recent studies of Reimann (1992) indicate that *Chenopodium* species like *C.album* and *C.schradericinum* are exceptions to this trend and they possess mechanism for sodium exclusion at root level.

In 1992, He and Cramer reported that at higher salinity levels Na concentration was increased 40-60 fold over that of control in all salt sensitive species of *Brassica* compestris, *B.juncea* and *B.nigra* while in salt tolerent *B.napus* Na accumulation was increased only by 29 fold.

Chavan and Karadge (1980) reported that in groundnut sodium concentration increases with increasing concentration up NaCl salinity. Number of workers also reported same results in different plants e.g. Gaikwad, 1995 in *Amaranthus* spp.; Varshney *et. al.* 1998 in *Cicer arientinum.*; Gadallah 1999 in *Vicia faba* and Esechie *et.al* .2002 in alfaalfa.

According to Kurban *et.al.* (1999) one of the leguminous plant *Alhagi* psedaalhagi can grow at very high salinity i.e. 900 m mol. Kg⁻¹ dry wt in 200 mM treatment compared to 20 mm ol. Kg⁻¹ in control.

Dubey *et.al.* (2008) reported that in *Citrus* sodium contents in leaves, stem and roots increases with increasing levels of NaCl in all genotypes of varying degree. According to Nikam and Chavan (2008) in *Chlorophytum borivilionum* accumilation of Na was more in leaf tissue than tuber tissue due to salt stress.

Table 7.1

Effect of NaCl on Inorganic constituents in C. album

Sr. No.	NaCl concentration	Na	K	Ca	Mg	Fe
1	Control	1.1	3.9	5.3	2.6	1.8
2	4 EC	1.5	3.4	1.9	2.7	1.6
3	8 EC	1.7	2.9	2.2	3.4	1.5
4	12 EC	1.9	3.4	1.8	3.0	1.5

Values expressed in g/100g dry tissue



b) The effect of Distillery effluent on Na content in C-album :

Distillery effluent is directly analysed for Na, Ca, Mg, Fe, P, volatile acid, the results are depicted in table 7.2a. It is observed that Ca, Na, Cl are more in distillery effluent. The result of the effect of distillery effluent on sodium concentration in

C.album are recorded in table. 7.2b and fig, from fig it is clear that Na decrease with increasing concentration of distillery effluent. At 80% concentration sodium content is slightly increases but it is less than control.

Roy *et.al.* (2008) studied the effect of petrochemical LTD effluent on the *Oryza* sativa and they observed that lower nutrient uptake by rice plant with increasing oil content in the soil.

According to Kumar in *Hordeum vulgare* Var.IB65(2001) sodium contents were increased in plant if distillery effluent used for irrigation purposes. Somshekar and Siddaramih (1997) reported that the accumulation of the metals in the soil and plant takes place by application of heavy metal rich industrial effluent. Bumbla *et.al.* (1968) also reported that inhibition of seed germination and growth due to high salt concentration in effluent.

Jamale (1987) studied the effect of distillery effluent on inorganic concentration of *Phaseolus aureus* and he reported that Na content decreases up to 25% distillery effluent treatment, however it increases markedly at 50% and above 50% concentration of distillery effluent treatment.

Ta	b	le	7	.2a
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Sr	Parameter	Value in ppm
1	P ^H	7.9
2	Total ash	0.64
3	Calcium	1803.6
4	Magnesium	487.29
5	Chlorides	9900
6	Nitrogen	950.6
7	Phosphorous (as P205)	183.2
8	Potassium (as K2O)	2168.28
9	Sodium	800
10	Iron	73.05
11	Volatile Acid	32.91

Characteristics of distillery effluent (Values expressed in ppm)

Table 7.2b

Sr. No.	Distillery effluent concentration	Na	К	Ca	Mg	Fe
1	Control	1.6	3.5	2.0	3.2	0.2
2	10%	1.5	3.0	2.7	4.7	0.2
3	20%	1.3	3.0	3.2	2.8	0.3
4	40%	1.3	1.9	1.5	2.2	0.1
5	60%	1.1	4.0	1.8	2.2	0.3
6	80%	1.4	2.1	2.7	2.4	0.2

Effect of distillery effluent on Inorganic constituents in C. album

Values expressed in g/100g dry tissue

Inorganic constituents



c) The effect of Vermiwash on Na content in C.album:-

Vermiwash is directly analysed for Na, Ca, Mg, Fe, P, volatile acid, the results are depicted in table 7.3a. It is observed that Ca, N, K, Mg, Fe and Cl are more in vermiwash. The results of vermiwash on sodium content in *C.album* are depicted in

table 7.3b and fig. It is evident that sodium contents are decreases with increasing concentration of vermiwash up to 80%.

According to Sivasubramanian and Ganeshkumar (1999) vermiwash application stimulate the growth and yield, which increased in marigold. He also observed that vermiwash contains higher sodium than the other treatments like cow dung, cow urine and vermicompost.

In present investigation we have analysed the content of sodium in vermiwash. It is 800 ppm and the application with proper dilution of vermiwash gives good results.

According to Sindhumol (2008) vermiwash is diluted with water (10%) and sprayed on plants which acts as a good tonic for plants and as a pesticide.

Sr	Parameter	Value in mg/l
1	P ^H	8.2
2	Calcium	139
3	Chlorides	22
4	Iron	0.2276
5	Magnesium	17
6	Nitrogen	3.08
7	Potassium (as K2O)	17
8	Sodium	22
9	Phosphorous (as P205)	0.015
10	Volatile Acid	17.14

Table 7.3a

Characteristics of vermiwash (Values expressed in mg/l)

Joshi (1988) reported that Na content was slightly elevated in senescent leaves of mango and custard apple. Na level was decrease in the senescent leaves of guava and pomegranate.

Bhivare (1984) studied *P. Vulgaris* and indicated that high content of Na in the senescent leaves of the plant are correlated with increased hydration and decreased K content as it is distributed in photosynthetic carbon metabolism.

Ambika and Karmarkar (1975) reported that there was increase in Na content in senescent leaves of succulent plant *Kalenchoe pinnate*.

In 1996 Naidu and Swamy reported that the leaf sodium concentration increased slightly throughout the life span of leaves until senescence.

Table 7.4

Effect of leaf senescence on Inorganic constituents in *C. album* Values expressed in g/100g dry tissue

Sr.No.	Leaf stage	Na	К	Ca	Mg	Fe
1	Young	0.84	1.74	1.53	1.68	0.17
2	Premature	0.80	1.70	2.05	1.41	0.19
3	Mature	0.76	1.84	1.70	1.43	0.24
4	Onset of senescence	0.78	1.78	2.60	1.08	0.34
5	Senescent	0.82	1.64	3.60	1.03	0.40



Table 7.3b

Sr.No.	Vermiwash concentration	Na	К	Ca	Mg	Fe
1	Control	4.8	3.7	3.8	3.4	0.08
2	10%	4.2	5.2	4.2	3.6	0.36
3	20%	4.1	3.8	4.0	3.2	0.18
4	40%	4.0	3.7	3.8	3.1	0.15
5	60%	3.8	3.5	3.6	2.8	0.13
6	80%	3.3	3.3	5.8	2.6	0.14
7	100%	3.4	3.2	7.0	2.4	0.18

Effect of vermiwash on Inorganic constituents in *C. album* Values expressed in g/100g dry tissue



d) The effect of Senescence on Na content in C.album:

The results of senescence on sodium content in *C. album* are recorded in table 7.4 and fig, from fig it is evident that sodium content decreases from young to mature but it slightly increases in on set of senescence and senescent leaves that is sodium is accumulated in senescent leaves this may be due sluggish Na mobility in *C. album*.

Joshi and Mishra (1970) have observed an increase in sodium content in the senescent leaves of halophyte *Clerodendron innerme* plant. Hyder (1971) observed same result in *Citrus* leaves.

B) Potassium (K):

Potassium is monovalent cation required by plant for many metabolic processes and osmotic regulation Okenenko *et.al.*, (1978). It also acts as activator for numerous enzymes. According to Evans and Sorger (1966) potassium is also necessary for starch synthesis, glycolysis, oxidative phosphorylation, photophosphorylation and adenine synthesis.

According to Mengel and Kirkby (1982) potassium is very mobile in plant and the bulk of K is mainly taken up during the vegetative growth stage. It is mainly accumulated in the cytoplasm of plant cell.

a) The effect of NaCl Salinity on K content of C. album:

The results of NaCl salinity on potassium content of *C.album* are depicted in table 7.1 and fig, from this it is evident that K^+ content decreased upto 8EC NaCl concentration. K is higher in 12EC NaCl treatment but less than control.

Kumar (1984) also noticed that the accumulation of K^+ was higher in tolerant than in sensitive group of Indian mustard. According to Romero and Maranon (1996) NaCl salinity stress cause potassium decline from roots and was accumulated in the leaves and fruits of *Melilotus segetalis*.

According to Nikam and Chavan (2008) K⁺ uptake was found to be affected by salt stress in *Chlorophytum borivilianum*.

Potassium concentration increased due to salinity has been recorded in Eucalyptus species (Prat and Fathi Ettai, 1990) and in bean (Carbonell, Burrachina et.al.1997)

Gaikwad (1995) reported that K^+ content was increased in leaf and stem tissue of *Amaranthus caudatus* and *A. hypochondriacus* and decreases in root and panicle tissue.K was increased in all parts of *A. paniculatus* with increasing salinity. Tozlu *et.al.* (2000) reported among other nutrients K^+ was affected the most in response to salinity. It was decreased in the root tissue of *Panicrus trifoliate* but was increased in the leaf tissue with increasing salinity.

b) The effect of Distillery effluent on K content in C.album:

The results of distillery effluent on potassium content in *C.album* are shown in table 7.2 and fig., it is clear that potassium content is decreased up to 40% but it is higher in 60% distillery effluent treatment and again it decline slightly.

Kumar (2001) studied the effect of distillery effluent on chemical constituents of soil and *Hordeum vulgare* var. IB65 and reported that there was reduction in potassium, total nitrogen, carbohydrate and crude protein.

According to Jamale (1987) in *Phaseolus areas* K^+ content was increased with increasing concentration of spent wash. Neutralized distillery effluent did not affect soil pH but increase the exchangeable potassium.

c) The effect of Vermiwash on K content in C.album:

The result of vermiwash on potassium content in *C.album* are depicted in table 7.3. It is clear that K^+ content is higher in 10% vermiwash treated leaves than any other concentration of vermiwash. All other concentration of vermiwash spray show more or less equal quantity of K^+ in *C.album* leaves.

According to Vijaya *et.al.* there was significant increase in the growth and yield in vermicompost treated plants *Cyampsis tetragonoloba* over control. The inorganic contents were also increased in the soil amended with vermicompost i.e. K⁺, N, P were increased as compare to untreated soil and plant.

d) The effect Senescence on K content in C.album:-

The results of leaf aging on potassium concentration of *C. album* are recorded in table, 7.4 It is evident that potassium content is higher in mature leaves as compare to other stages of leaf. K is decreased in senescent leaves as it is very mobile.

Koo and Young (1977) studied the effect of age on mineral composition of mango leaves and they found decrease in potassium content with advancing age of leaves.

Patange and Patil (1980) reported that higher levels of potassium in younger leaves of Jambhul. In 1981 Thakur *et.al.* observed decrease in potassium in senescent leaves.

Bengits Som *et.al.* (1983) studied uptake and distribution of potassium in cucumber leaves of different age and noticed that K^+ concentration decreased with increasing leaf age.

Joshi (1988) reported that mature leaves of pomegranate, mango, custard apple and guava have the highest level of potassium and its concentration was decreased in senescent leaves of all trees.

C) Calcium :-

Calcium is essential in the synthesis of pectin in the middle lamella of the cell wall. It plays an important role in the regulation of membrane permeability of various ions, in particular to inorganic cations (Van steveninck) .It also plays an important role in the metabolic processes. Mengel and Krikby, (1982). Ortiz *et.al.* (1994) reported that Ca enhances the net absorption of K. According to Cleark (1984) the activities of many enzymes have been either stimulated or inhibited by calcium. According to Swami and Raddy (1991) calcium also maintains levels of superoxide dismutase and catalase which may be controlling the lipid peroxidation.

a) The effect NaCl Salinity on Ca in C.album:-

The results of NaCl salinity on Ca concentration in *C.album* are recorded in table 7.1 and fig, it is evident from the result that calcium contents are the highest in mature leaves and Ca contents decreased as the effect of increasing NaCl salinity. Singh and Yadav(1999)in *Ponamia pinnata* Ca contents decreases with increasing salinity. Essa (2002) also observed same results in three Soyabean cultivars. Our results are on the similar lines. Meiri *et.al* (1971) have observed reduction in Ca uptake in bean plant due to NaCl and Na₂SO₄ salinization. Imamul Hug and Larher (1984) and Cramer *et.al.* (1987) have also reported that salinity hampers Ca uptake in salt sensitive plants.

According to Chakrabarti and Mukherji (2002) in various parts of mungbean show decreased calcium content under NaCl stress.

b) The effect of Distillery effluent on Ca contents in C.album :

The results of distillery effluent on Ca contents in *C.album* are shown in table 7.2, from the result it is clear that calcium contents are increased with increasing

concentration of distillery effluent. There is slight decrease in calcium concentration at 40 and 60% distillery effluent treatment.

According to Kumar(2001) in *Hordeum vulgare* var IB65 calcium, sulphate, Chloride and ash content of root, stem, leaf and seeds were increased by the treatment of distillery effluent.

According to Jamale (1987) calcium content in *Phaseolus* aureus treated with spent wash showed markedly increase at higher concentration of distillery effluent (i.e. above 60%)

Lokhande and Bhosale (1983) reported that Ca and P increased in *Dichanthium annulation* due to distillery effluent treatment.

c) The effect Vermiwash on Ca contents in C.album:

Effect of vermiwash on calcium contents of *C.album* has been studied and the results are shown in table 7.3 and fig., the result indicate that calcium contents increased with increasing concentration of vermiwash treatment.

According to Vijaya *et.al.* (2006) effect of vermicompost on soil and plant *Cyamposis tetragonoloba* showed that there was enhanced amount of calcium in soil, it was because of the activity of calciferous glands which absorb excess Ca from their diet, from which it is excreted via the digestive trach.

Sivasubramanian and Ganeshkumar (2004) studied the effect of foliar spray of vermiwash on marigold. He observed that vermiwash spray enhanced the growth and yield as compared to other treatment and control. He also reported that vermiwash contain higher amount of calcium concentration as compare to other contents.

In the present investigation Ca contents are increased as a result of vermiwash treatment.

According Springett and Syers (1979) earthworms altered nutrient availability, altered the plants ability to take up nutrient or affected the growth mechanism of the plants.

d) The effect of senescence on Ca contents in C.album:

The results of leaf senescence on calcium content of *C.album* are recorded in table 7.4, from results it is clear that calcium is increased from young to senescent stages of leaf.

Amonkar and Karmarker (1978) reported that calcium content is relatively more in senescent leaves than mature or young leaves of *Salvadora persica*.

According to Naidu and Swamy (1996) the concentration of calcium increased throughout the life span. In Zea mays Clerk (1974) observed a considerable increased in calcium concentration in aging leaves. Waughman and Bellamy (1981) noticed accumulation of Ca in senescent leaves of several plant species. Hazel and Halliwell (1984) reported that in pea plant (*Pisum sativum*) Ca content increased with age of plants.

Salunkhe (1997) reported that increase in calcium content in senescent leaves of *Ipomoea cornea*. The results of the present investigation also showed increase in Ca contents in senescent leaves of *C.album*.

D) Magnesium:

Magnesium is a mobile and strongly electropositive divalent cation in the plants. It is present in the structural formula of chlorophyll molecule. It plays an important role in activation of enzymes, involved in most of the metabolic reactions e.g. respiration, photosynthesis and the synthesis of DNA and RNA.

According to Clark (1984) in some circumstances Mg may contribute to the electrical neutrality of organic compounds such as sugar phosphates, sugar nucleotides as well as amino acids. Its deficiency shows chlorosis and necrosis of leaves.

a) The effect of NaCl on Mg content of C.album:-

The results of the effect of NaCl salinity on the Mg contents of *C.album* leaves are recorded in table 7.1. The results indicated that Mg contents increased as NaCl salinity increases. These results are confirmally with the chlorophyll increase due to NaCl salinity.

Cordovilla <u>et.al.</u> (1995b) in *Glycine max* and *Phaseolus valgaris*, reported that Mg content increases due to salinity. According to Darger *et.al.* (2004) in *Salvadora persica* Mg content increase with salinity.

According Nikam and Chavan (2008) salt stress caused an increase in calcium and magnesium contents in both leaf and tuber tissue of *Chlorophytum borivilianum*.

According to Delfine *et.al.* (1998) the decreased Mg levels in the leaves of spinach due to salinity might be responsible for decrease in chlorophyll content. Immamul Hug and Larher(1983) observed similar results in *Phaseolus aureus*.

In 2005 Rabie *et.al.* reported that in cowpea Mg content decreased with increasing salinity. Naeini *et.al.* (2004) reported similar observation in *Punica* granatum.

According to Katony (2007) salinity promoted uptake and transport of Mg but its accumulation in *Linum usitatissimum*, most of the Mg taken up was transported to the aerial parts of the plant and allocated to the stems on the other hand, salinity retarded uptake and transport of Mg by *H. cannabinus*.

b) The effect of Distillery effluent on Mg contents in C.album:

The results of the effect of distillery effluent on the concentration of Mg in C.album are shown in table 7.2 and fig. It is evident from the result that Mg contents decreased with increasing concentration of distillery effluent.

According to Pragassam and Kannabiran (2001) in *Vigna mungo* all parameters were increased over control due to distillery effluent treatment.

According to Naidu (2002)the 4 categories at plant symptoms to oil pollution, typical of nutrient deficiencies include yellow foliage, chloronecrosis, wilting and defoliation and leaf roll.

According to Sharma *et.al.* (2002) higher concentration (>5%) of effluent were found to be toxic, however it is beneficial when used in proper dilution.

According to Singh (1982) the inhibition of photosynthetic activity in legumes particularly reduction in chlorophyll contents may be due to the osmotic effect of high effluent concentration which reduce the uptake of Mg++ and other mineral ions which leads to inhibition of photosynthesis.

Sohni (1994) reported higher chlorophyll content in the forest seed beds treated with lower concentration of distillery effluent.

According to Behera and Misra (1983) RNA and Chlorophyll contents of the seedlings were found most susceptible to effluent. He also observed that loss in hill reaction activity.

Ronu Roy (2008) observed that increased effluent concentration may denature the chloroplast protein which subsequently affect the photosynthetic apparatus hence it also affect the magnesium content of plants. Our results of Mg decreased due to the effect of distillery effluent which is toxic and affecting chlorophyll and Mg released is exported some where else.

c) The Effect of Vermiwash on Mg contents in C. album:-

The effect of vermiwash Mg contents of *C.album* has been shown in table 7.3. It clearly shows that Mg contents are increased with increasing concentration of vermiwash spray.

According to Savithramma *et.al.* (2006) due to addition of vermicompost in the soil it improves the physical properties of soil and also improves chemical properties of soil such as pH, N, P, K^{T} , Ca and Mg.

The Mg concentration in vermicompost applied to soil is increased due to higher concentration of exchangeable Mg in the worm cast than the surrounding soil.

Sivasubramanian and Ganeshkumar (2004) observed that Vermiwash contains more amount of Mg as compared to other treatments (Cow dung, Cow urine, vermicast), hence due to Vermiwash spray on marigold plant growth was increased and maximum number of leaves per plant were produced.

In the present investigation we also observed that vermiwash contain 487.29 ppm magnesium. Hence it is beneficial for the growth of *C.album*.

Gajalakshmi *et.al.* (2002) observed that application of Vermicompost leads to significant improvement on the growth and flowering as compared to the untreated plants.

d) The effect of Senescence on Mg contents in C.album:

Effect of senescence on the Mg contents has been studied in *C. album* and the results are depicted in table 7.4 and fig. It is clear that Mg contents in the senescent leaves are decreased as compare to mature and young leaves. This is due to breakdown of Chlorophylls and transport of Mg from senescent leaves to younger plant parts.

According to Hebber *et.al.* (2008) in wheat germplasm Mg content was accumulated more in green leaves. Waghaman and Bellamy (1987) studied Mg^{++} levels in mature and old leaves of 21 plants species and they noticed a lowering of Mg^{++} contents in aged leaves. They also noted that in *Hordeum vulgare* L. Mg^{++} content was decline i.e. mature leaves contained 3 to 5 times more Mg^{++} than old leaves.

Salunkhe (1997) also reported that Mg++ contant decreased in the senescent leaves of *Ipomea* spp. Our results of the present investigation are on the similar lines in *C.album*.

E) Iron:

Iron has an important biological significance among the various micronutrients. Iron is present in flavoproteins, Cytochromes and several enzymes. In living cell it mainly occurs in porphyrin or hemes. Fe is required for stability and activity of many enzymes. According to Machold and Stephan (1969) iron is essential for biosynthesis of chlorophylls. It is required for the formation of protochlorophyllide from Mg-protoporphyrin. Due to Fe deficiency chlorosis and several other problems in aerobic respiration can be occurred.

a) The effect of NaCl on Fe contents in *C-album*:

Effects of NaCl salinity on Fe contents has been studied in *C. album*. It is evident from the results recorded in Table 7.1 that Fe contents decreased with increasing NaCl salinity.

In 2003 El- Hamdaoui reported that salinity caused to reduce the iron content in pea plant. According to Hassanein (1999) in rice seedlings Fe contents decreased with increasing NaCl salinity. Shimose (1972) has studied iron uptake in crops like barley, wheat and *Asparagus* under salt rich environment and concluded that pattern of iron uptake differs from species to species. In 1981 Karadge and Chavan observed that in peanut Fe content increased with NaCl salinity, Carbonell Barrachina *et.al.* (1998) reported similar observation in bean.

According to Delgada and Sanchez Raya (1998) in sunflower Fe content decreases with NaCl salinity.

b) The effect of Distillery effluent on Fe contents in C. album:

The effect of distillery effluent on Fe content has been studied in C. album and the results are recorded in table 7.2. The results indicate that Fe content is increased in 10% and 20% concentration. The Fe contents increases in 60% and 80% However Fe content is decreased in 40% concentration at distillery effluent treatment.

According to Somshekhar and Siddaramaih (1997) by the application of heavey metal rich industrial effluent there was accumulation of the metals in the soil.

According Siddaramaih (1998) growth of chilli was reduced and also reduction in all parameters when treated with heavey metal rich industrial effluent.

According to Monomani *et.al.* (1990) the minralization of soil organics lead to increase in the amount of NPK and other metal ions. Due to alcohol and chemical industry effluent.

Naidu (2002) reported that certain plant showed symptoms like yellow foliage, chloronecrosis, wilting, defoliation and leaf roll due to oil pollution.

According to Veronica Migo *et.al.* (2008) there was increased yield of sugar cane when fertilized with distillery effluent in proper dilution. They also noted that the results indicate that distillery effluent alone can substitute for the recommended rate of 175 kilogram Nitrogen per hectare and can supplement 10-24 kg of phosphate per hectare and 800-100 kg of K⁺ per hectare as well as nutrients Ca, Mg, Fe, Mn and Zn.

c) The effect of Vermiwash on Fe contents in C.album:

Effect of vermiwash on Iron content in *C.album* leaves was studied and results are recorded in table 7.3, the results are indicating that the iron content is increased in 10% and 6.% concentration of vermiwash as compared to other concentration of vermiwash.

According to Vijaya *et.al.* (2006) the iron content was increased in the soil treated with vermicompost. They also reported that the higher concentration of Cu and Fe attribute to humic acid like components in vermin compost.

Aina (1984) reported that earthworms can improve soil by increasing soil porosity, water infiltration and water holding capacity.

Sivasubramanian *et.al.* (2004) reported that in marigold vermiwash treated plants showed more growth and yield as compare to other treatments.

We also analyzed the Fe content in vermiwash and found out that vermiwash contain 0.2276 mg/l iron (Fe).

d) The effect of senescence on Fe contents in *C-album*:

The effect of senescence on the iron contents is investigated in C.album leaves and results are shown in table 7.4. From results it is evident that iron is accumulated in the senescent leaves. Iron contents increased from young to senescent stage of leaves of C.album.

According to Joshi (1988) iron is accumulated in pomegranate senescent leaves as compare to other 3 fruit species where iron was decline in senescent leaves.

Woughman and Bellamy (1981) reported that in perennial species iron was increased in the senescent leaves.

Tanaka et.al. (1966) noticed that iron was deposited in the older leaves of some crop species like rice.

Chavan and Patil (1980) reported that Fe concentration increased in aged leaves of Achars sapota during different stages. They also reported that in Achras sapota and Suzygium cumini depletion of iron from the leaves during fruit development.

8. Enzyme studies in C.album:

A) Nitrate reductase (N.R.):

According to Beevers and Hagemen (1969) nitrates are the most abundant nitrogen source available for the plants. The reduction of nitrates to nitrites and subsequently to ammoniam by plants takes place in the cytoplasm and plastids. This reduction of nitrate takes place with the help of two enzymes nitrate reductase (NR) and nitrite reductase (NiR).

Nitrate reductase is a metaloflavo-protein that catalyzes the reduction of nitrate to nitrite which is important in first stage of protein synthesis. Nason and Evans (1953) firstly isolated nitrate reductase from *Neurospora*. According to Shrivastava (1981) the enzyme is regarded as a rate limiting in nitrogen assimilation process in higher plants. Sinha and Nicholas (1981) reported that NR activity generally inhibited by extreme external conditions such as extreme water, salt and heat stress.

a) The effect of NaCl Salinity on Nitrate Reductase (NR) in C.album:

The effect of NaCl salinity on the rate of NR in *C.album* is studied and the results are recorded in the table 8.1 and fig. It is clear that the NR activity is decreased (inhibited) in increasing NaCl salinity.

According to Khan *et.al.* (1997) in *Medicago sativa* the NR activity decreases with increasing NaCl salinity due to substantial reduction in nitrate contents. Garg *et.al.* (1997) reported that the activity of N.R. in cluster bean was inhibited by salinity stress.

Khan (1994) noted in pea, lentil and Chickpea that nitrate reductase activity has been decreased under saline conditions. While Chavan (1980) reported that the increase in enzyme NR activity enables the plant to cope with changes in nitrogen metabolism induced by salinity. Krishnamoorthy and Siddique (1985) also noted that nitrate reductase activity can be stimulated due to salinity.

Austenfeld (1974) observed that stimulation of NR at 50 to 150 mm NaCl and inhibition at higher concentration i.e. at 250 mM NaCl in *Chenopodium album*. Garg *et.al.* (2005) also reported that in sesame NR activity was decreased with increasing NaCl salinity.

In 2004 Maighany and Ebrahimzadch noticed in wheat plant that NR activity was affected more under salt stress. They also reported that the inhibition of its activity was dependent on the cultivars, organs development stage and concentration of salinity treatment.

Table 8.1

Sr.No.	NaCl concentration	NR activity in h ⁻¹ g ⁻¹ fresh plant
1	Control	1.184
2	4 EC	0.932
3	8 Ec	0.808
4	12 Ec	0.858

Effect of NaCl salinity on the activity of Nitrate reductase in C. album



b) The effect of Distillery effluent on NR activity in C.album:

The results of the effect of distillery effluent on NR activity in *C.album* leaves are shown in table 8.2. From results it is evident that NR activity decreases with increasing the distillery effluent concentration. However the NR activity seems to be stimulated and show increase at the 40% distillery effluent concentration.

According to Behera and Mishra (1985) in rice seedlings the activity of all enzymes tested except succinate dehydrogenase, decreased during effluent treatment. Pragasam and Kannabiran (2001) reported that in *Vigna mungo* all the parameters were increased over control and 10% and 25% concentrations but they were reduced at higher concentration.

According to Agarwal and Pandey (1994) spentwash has high capability of extracting out Mn^{2+} soil which is made available to the plant very easily. If there is deficiency of Mn^{2+} in soil it results into decrease in functioning of some oxidative enzyme as well as functioning of oxygenous and carboxylase group of enzymes.

Sr.No.	Distillery effluent concentration	NR activity h ⁻¹ g ⁻¹ fresh leaf tissue
1	Control	1.448
2	10%	1.158
3	20%	1.078
4	40%	1.770
5	60%	1.393
6	80%	0.885

Table 8.2

Effect of distillery effluent on Nitrate reductase activity in C. album



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c) The effect of Vermiwash on NR activity in C.album:

The effect of vermiwash on the activity of NR in *C.album* leaves is undertaken in the present investigation and the results are depicted in table 8.3. It is clear that the activity of enzyme nitrate reductase is decreased at all concentrations of vermiwash.

Table 8.3

Effect of vermiwash on nitrate reductase activity in C. album

Sr. No.	Vermiwash concentration	NR activity in h ⁻¹ g ⁻¹ fresh tissue
1	Control	1.708
2	10%	1.706
3	20%	0.638
4	40%	0.646
5	60%	0.644
6	80%	0.762
7	100%	0.768

(h ⁻¹ g ⁻¹	fresh	tissue)
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The result of the effect of senescence on the activity of NR are shown in table 8.4 and fig. from table it is evident that nitrate reductase activity decreases

from young to senescent stage of leaf. Activity of NR is highly decreased in senescent leaves.

Nicholes *et.al.* (1976) reported that in soyabean NR activity was the lowest in the oldest leaf tissue and the highest in the youngest trifoliate leaves.

Linn and Rao (1980) observed that younger but physiologically mature leaves of *Triticum* have higher nitrate reductase activity than the older leaves.

According to Munjal *et.al.* (1983) in Winged bean (*Pseocarpus tetragonolobus* L) NR activity was higher in fully developed young and physiologically mature trifoliate leaves than that mature and senescent leaves.

In 1988 Joshi also reported that nitrate reductase activity was higher in young developing leaves of Custard apple, Mango and Guava than the senescent leaves.

Table 8.4

Effect of senescence on Nitrate reductase activity in C. album

Sr.No.	Leaf Stage	NR activity in h ⁻¹ g ⁻¹ fresh tissue
1	Young	1.498
2	Premature	1.162
3	Mature	0.818
4	Onset of senescence	0.504
5	Senescent	0.058

(h⁻¹g⁻¹ fresh tissue)



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B) Study of Enzyme Catalase in C.album:

Catalase is one of the most important catalysts which catalyses conversion of H_2O_2 , a powerful and potential harmful oxidizing agent to H_2O and O_2 . As catalase protects the cells from being destroyed by H_2O_2 it forms major component of cellular mechanisms of defense against oxidative stress. Grinberg (1971) has suggested that since catalase has got more affinity for H_2O_2 it is mainly involved in H_2O_2 breakdown in plant tissue. Catalase is a heme containing redox enzyme. Each molecule of catalase is a tetramer of 4 polypeptide chains. Catalase catalyses H_2O_2 and also acts as hydrogen acceptor.

a) The effect of NaCl Salinity on the activity of catalase in C.album:

The results of the effect of salinity (NaCl) on the activity of catalase in *C.album* leaves are shown in table 9.1. It is clear from the result that catalase activity decreases with increasing NaCl salinity.

According to Saha and Gupta (1997) catalase activity was decreased under salinity treatment in sunflower. In 1999 Saha and Gupta also reported similar observation in Mungbean. In 2004 Cavalcanti *et.al.* observed that the catalase activity in the leaves of cowpea plants, experienced a two fold decrease only after 1 day NaCl treatment and further the salt withdrawal had not effect on its recovery.

In 2004 Manikanandan and Venkatesan reported that the catalase activity was increased up to optimum level due to salinity treatments and there after decreased in *Aegiceros cornicalatum*.

According to Sharma *et.al* (2008) in *Jatropha curcus* L. leaves catalase activity increased with increase in soil salinity Mishra and Das (2004) reported similar observation in *Bruguiera parviflora*.

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Sr.No.	NaCl concentration	Catalase activity (mg ⁻¹ protein min ⁻¹)
1	Control	0.050
2	4 EC	0.039
3	8 EC	0.024
4	12 EC	0.022

Effect of NaCl salinity on Catalase activity in C. album

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b) The effect of senescence on the activity of enzyme catalase in C.album:

The effect of senescence on catalase activity in *C.album* leaves has been studied and the results are recorded in table 9.2. It is clear from the results that catalase activity is increased at mature stages of leaf as compared to younger ones. Then the activity is decreased in senescent leaves.

Paric (1968) observed decrease in catalase activity during senescence in detached leaves of tobacco.

Patra *et.al.* (1978) also noticed a decrease in catalase activity during senescence of several species.

In 1975 Kar and Mishra reported that the activity of catalase decreased during senescence of both attached and detached rice leaves. Mukherjee and Choudhari (1980) also reported similar observation in *Vinga* seedlings.

Pistelli *et.al.* (1992) reported that in *Beta vulgaris* leaves catalase activity decreased during the leaf senescence. Farkas *et.al* (1964) also showed similar observation in tobacco leaves.

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Effect of senescence on catalase activity in C. album (mg⁻¹ protein min⁻¹)

Sr.No.	Leaf stage	Catalase activity		
1	Young	0.021		
2	Premature	0.028		
3	Mature	0.034		
4	Onset of senescence	0.032		
5	Senescent	0.027		



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