CHAPTER - IV

RESULTS AND DISCUSSION

A. SEED GERMINATION AND GROWTH PERFORMANCE:

1. Germination Percentage :

The effect of seed treatment with Mancozeb on seed germination recorded in *Glycine max* (L.)Merr. after 24 h, 48 h and 72 h is depicted in Table 3 and fig. 1.

Table 3 : Germination percentage in *Glycine max*(L.)Merr. under the influence of Mancozeb.

Pesticide	Exposure period					
	6 h			12 h		
	Germination percentage after					
	24 h	48 h	72 h	24 h	48 h	72 h
Control	90	100	100	90	90	100
Mancozeb (% w/v)						
0.25	100	100	100	90	90	100
0.5	100	100	100	100	100	100
1.0	90	90	100	80	80	100
2.0	80	90	100	70	70	80

Values are mean of three determinations.

A stimulation in germination percentage was observed with lower concentrations of Mancozeb over the control. Inhibition of germination was noticed above 1 % concentration. A delay in the seed germination was observed in the seeds treated with higher concentrations of Mancozeb. The exposure periods used for the study did not affect the germination percentage upto 1 % of seed treatment. While higher concentrations caused marginal reduction.

A negative effect of fungicidal treatment on germination was commonly observed in a number of studies. Fungicide Mancozeb 75 % reduced both germination and coleoptile length in wheat and barley (Kollmorgan and Ballinger, 2003) . Detrimental

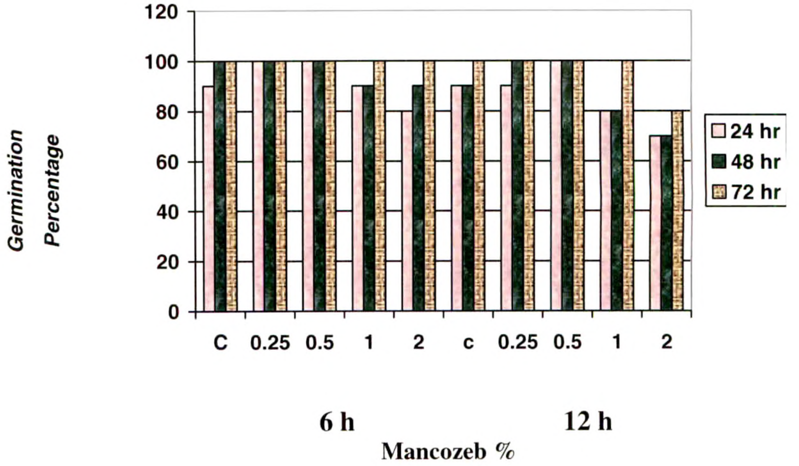


Fig. 1 : Germination percentage in *Glycine max*(L.) Merr. under the influence of Mancozeb

effect of benomyl have been reported in onion (Shehaby and Beaver,1991). Randhava et al. (1989) did not found enhancement on germination of wheat with seed treatment of captan, vitavax and thirum. In *Vigna,* carbendazim treatment affected seed germination (Chandra and mathur,1985 ; Somashaker and Rao, 1984).

There are few reports of positive influence of fungicides on plants. Khare (1976) reported beneficial effect of bavistin and thirum on germination and vigour of *Glycine max* L. Merr. Singh and Setia (1974) observed similar results in *Glycine max* L. Merr. when treated with thirum followed by bavistin. Pardeshi et al. (1989) also reported stimulatory effect of seed treatment with thirum followed by bavistin on germination and vigour of soybean. No significant effect of Bavistin - WP has been reported in *Phaseolus mungo* (Neelamegram and Sreelaja, 2006). Kar and Gupta (1993) obtained better germination in paddy with the treatment of bordeaux mixture, blitox, zineb and captan. From the above discussion, it is clear that the treatment of fungicides with low concentrations stimulate the seed germination.

concentrations of Anth (Chlorpyriphos + Cypermethrin) used in the present study (Table 4 and fig. 2). The higher concentration treatment of insecticide caused a significant reduction and delay in germination over control. Germination was greatly reduced and delayed by 12 h exposure period and higher concentrations of Anth (Chlorpyriphos + Cypermethrin).

Table 4 : Germination percentage in *Glycine max*(L.)Merr. under the influence of Anth (Chlorpyriphos + Cypermethrin)

Pesticide	Exposure period					
	6 h			12 h		
	Germination percentage after					
	24 h	48 h	72 h	24 h	48 h	72 h
Control	90	100	100	90	100	100
Anth(Chlorpyriphos + Cypermethrin) (% v/v)						
0.25	80	100	100	70	90	100
0.5	80	100	100	70	90	100
1.0	70	90	100	60	80	90
2.0	60	80	100	60	80	80

Values are mean of three determinations.

The inhibitory effect of insecticides on seed germination are reported in several studies. N.K. Singh (1998) observed inhibitory effect of dimethoate and phorate on germination and growth of wheat and rice. Khan et al. (2000) recorded inhibitory effect of endosulfan on germination and growth of fenugreek. Similar results were recorded in *Vigna mungo,* fenugreek, mustard and *Pisum sativum* with the treatment of rogor, BHC, DDT, nuvacron, gaucho and apron (Benjamini,1986 ; Mitra and Raghu, 1989 ; Verma et al. 1994 ; Kamble and Sabale 2001 ; Murthy and Leelavati,2001). Saraswathi et al. (1996) reported deleterious effect of sumicidin on germination of soybean.

al. 1994 ; Kamble and Sabale 2001 ; Murthy and Leelavati,2001). Saraswathi et al. (1996) reported deleterious effect of sumicidin on germination of soybean.

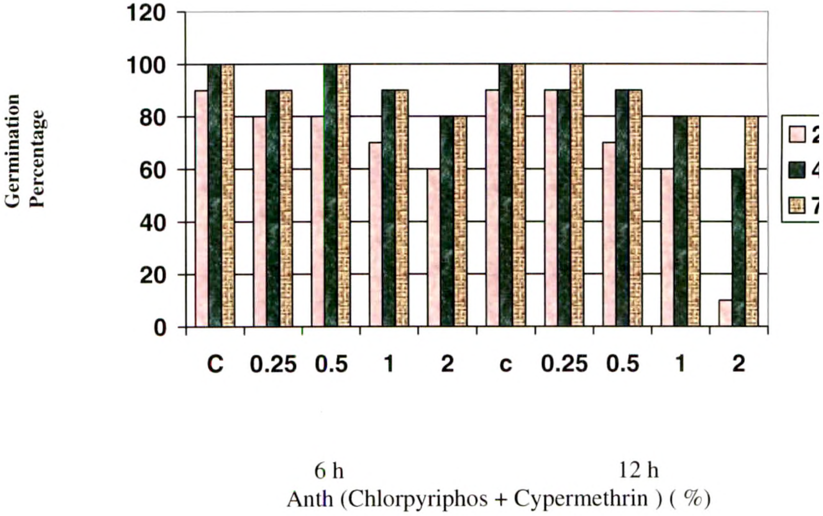
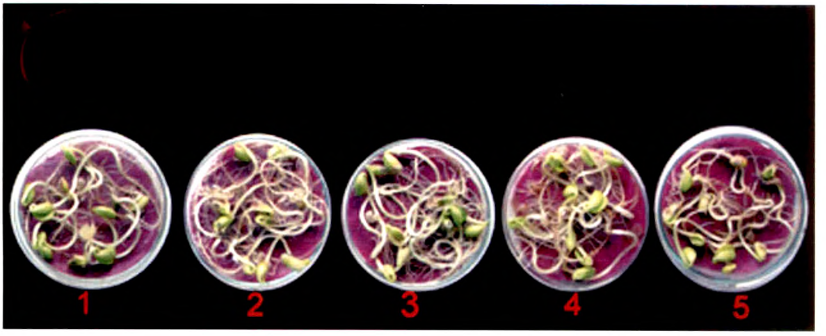
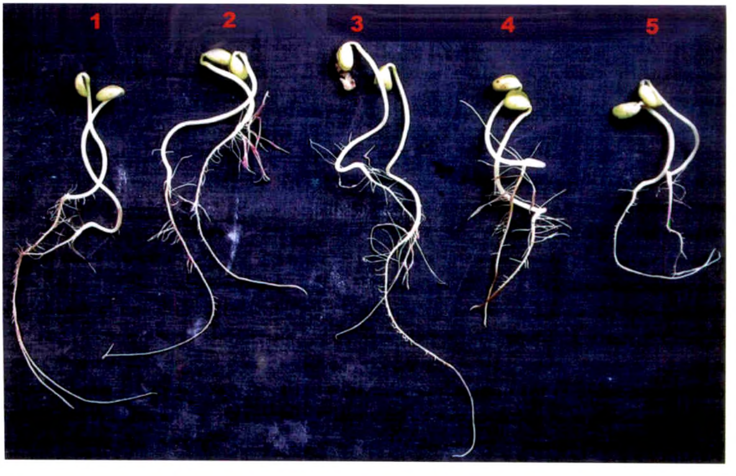


Fig. 2 : Germination percentage in *Glycine max* (L.) Merr. under the influence of Anth (Chlorpyriphos + Cypermethrin)

Patil (1997) reported stimulatory effect of lower concentrations of monocrotophos and monocrotophos in combination with bavistin on germination percentage of soybean. Gupta et al. (1998) noticed phytotonic effect in imidacloprid treated plots of cotton. Carbofuran, endosulfan and methyl parathion enhanced germination in sugarcane and Jowar (Sabale and Misal 1993 ; Sharma 1997). In the present work, negative response is

PLATE 1

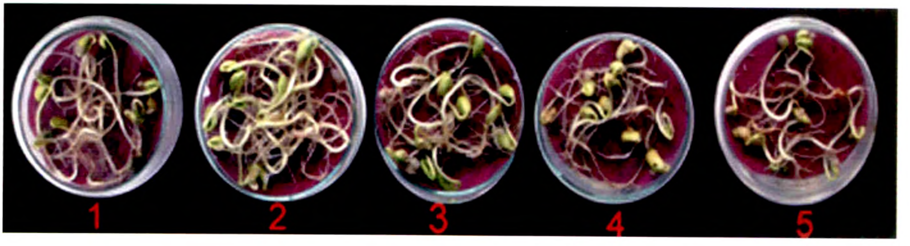


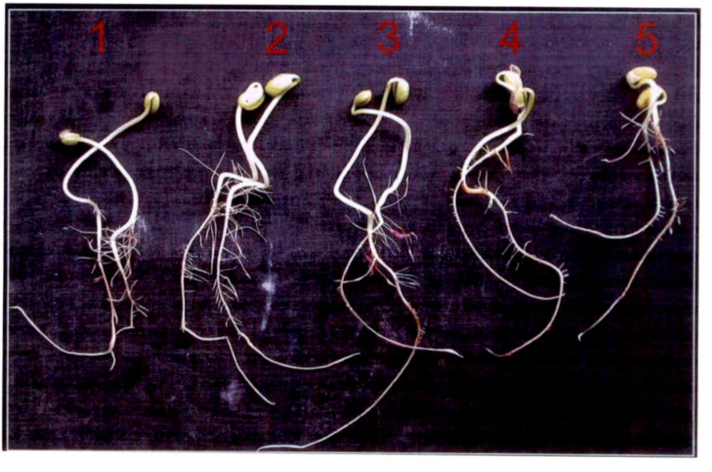


Influence of 6 h seed treatment of Mancozeb on seed germination and seedling growth of *Glycine max* (L) Merr.

1. Control, 2. 0.25 %, 3. 0.5 %, 4. 1 %, 5. 2 %

PLATE 2





Influence of 12 h seed treatment of Mancozeb on seed germination and seedling growth of *Glycine max* (L.) Merr.

1. Control, 2. 0.25 %, 3. 0.5 %, 4. 1 %, 5. 2 %

2. Seedling Growth :

Seedling growth of *Glycine max*(L.)Merr. in response to Mancozeb treatment has been presented in Table 5.

Table – 5 : Effect of Mancozeb on seedling growth *of Glycine max* (L.)Merrill.

Exposure Period	Concentrations (%w/v)	RL (cm)	SL (cm)	R/S	RLN	FW (g)	DW (g)	PP
6h	Control	4.96 ±2.43	4.67 ±1.14	1.06	5.84 ±1.94	o.41	0.12	-
	0.25	8.2 ±1.06	4.18 ±1.84	1.96	11.9 ±3.45	0.61	0.12	-65.32
	0.5	6.21 ±3.40	2.35 ±0.80	2.64	7.87 ±1.81	0.38	0.10	-25.20
	1.0	5.62 ±2.16	2.66 ±1.23	2.11	6.71 ±2.14	0.34	0.11	-13.30
	2.0	5.32 ±2.40	2.78 ±0.91	1.91	6.43 ±3.51	0.39	0.11	-7.25
12h	Control	5.53 ±1.73	2.7 ±0.60	2.04	4.81 ±2.23	0.40	0.12	-
	0.25	5.86 ±2.71	5.17 ±2.37	1.13	8.71 ±2.20	0.49	0.11	-5.96
	0.5	7.1 ±1.81	5.77 ±1.28	1.23	7.8 ±1.73	0.47	0.11	-28.39
	1.0	6.8 ±1.43	5.58 ±1.56	1.21	7.34 ±2.31	0.45	0.10	-22.96
	2.0	5.64 ±1.69	5.48 ±1.9	1.02	7.25 ±3.41	0.45	0.09	-1.99

SL- Shoot Length
RL- Root Length
FW-Fresh Weight
DW-Dry Weight
R/S- Root to shoot ratio
RLN-Rootlet Number
PP-Percent phytotoxicity.
Values are mean of ± S.D. based on three replicates.

At both the exposure periods i.e. 6 and 12 hour, stimulation in root length was observed at all the concentrations of Mancozeb. The seed treatment with 6 h exposure period of Mancozeb caused decrease in shoot length over control. While 12 h exposure period seed treatment positively influenced shoot length. All concentrations of Mancozeb at 6 h exposure period seed treatment positively influenced root to shoot ratio, while negatively affected at 12 h exposure period. For both the exposure periods, root to shoot ratio exceeded 1 due to negative effect on shoot growth as compared to root growth. Rootlet number increased by the seed treatment of Mancozeb with both the exposure periods. At 6 h exposure period seed treatment, only 0.25 % concentration of Mancozeb increased fresh weight. While all concentrations at 12 h exposure period increased fresh weight and dry weight over control.

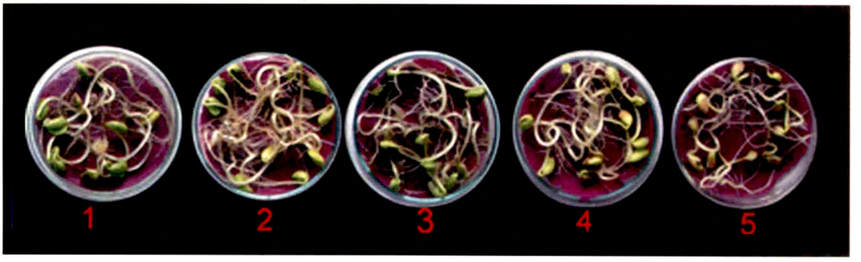
The phytotoxic effect of Mancozeb is well exhibited by the values of percent phytotoxicity. Negative values of phytotoxicity recorded for all concentrations and both exposure periods which indicated positive influence of Mancozeb seed treatment on seedling growth of *Glycine max* (L.) Merr.

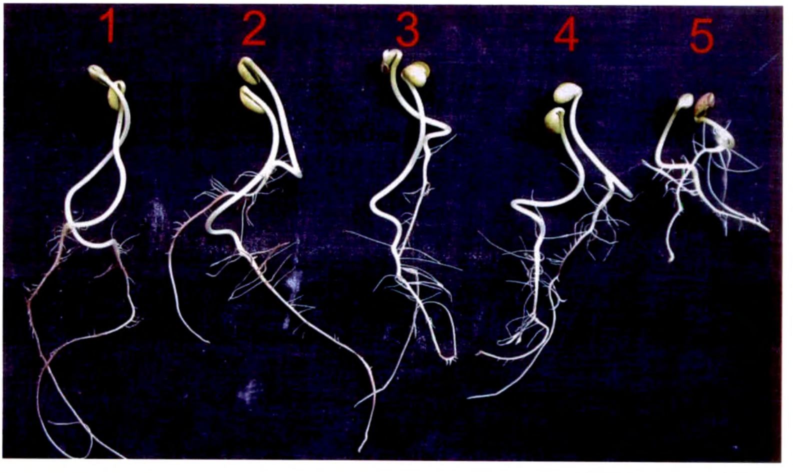
There are many reports stating beneficial effect of different fungicides on plant growth. The treatment of bavistin stimulated germination and growth in *Trigonella* at lower concentrations particularly the 12 hour exposure was found beneficial (Kamble and Sabale, 1999). Bavistin - WP treatment has no significant effect on seed germination of Blackgram. Seed germination was increased with increased concentrations of fungicide in 6 h treated seeds, while it was reversed in 24 h treated seeds (Neelamegam and Sreelaja, 2006). Captan, thirum, mancozeb, zineb, antracol and kitazin are known to influence seedling growth in *Dolichos biforus* L., *Raphanus sativus* L. and soybean (Reddi and Vidyavati,1983 ; Somashaker and Rao, 1984 ; Pardeshi et al. 1989).

The seedling growth under the influence of different concentrations and exposure periods of Anth (Chlorpyriphohs + Cypermethrin) is recorded in Table 6 .

In 6 and 12 hour exposure period seed treatment of Anth (Chlorpyriphos and Cypermethrin), the root length and shoot length increased with the treatment of 0.5 % concentration. Higher concentrations viz. 1% and 2% especially at 12 h exposure period significantly caused reduction in the root and shoot length. At 6 h exposure period seed treatment, all concentrations of Anth (Chlorpyriphos and Cypermethrin) positively influenced root to shoot ratio. While at 12 h exposure period seed treatment, higher concentrations are stimulatory to R / S ratio. In general, both the exposure periods and all the concentrations of Anth (Chlorpyriphos and Cypermethrin) caused increase in rootlet number except negative effect of highest concentration at 12 h exposure period. All concentrations of Anth

PLATE 3

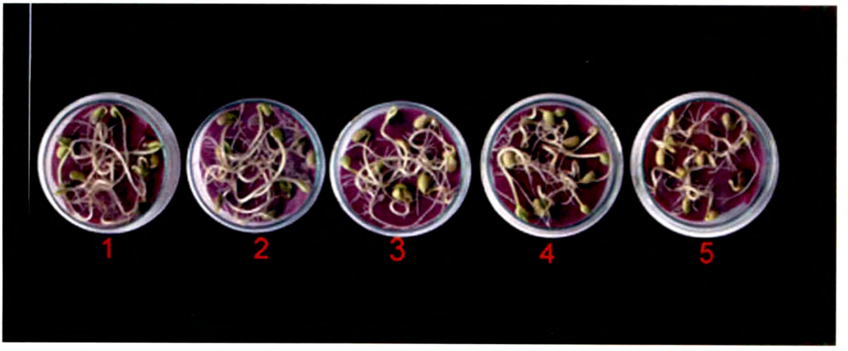


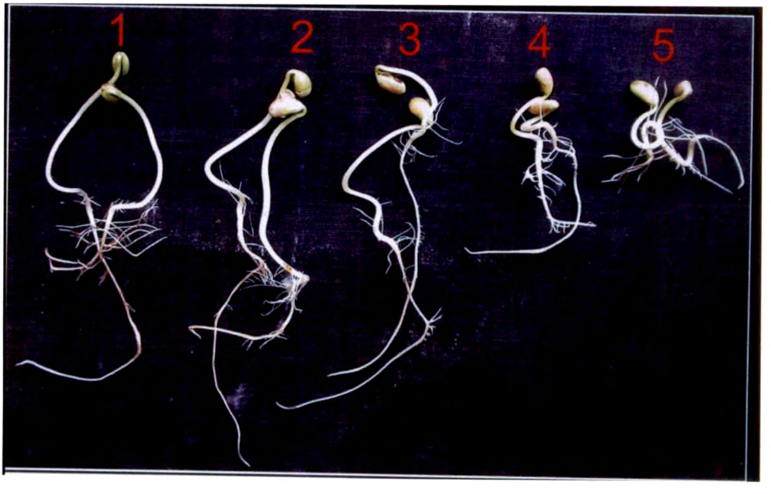


Influence of 6 h seed treatment of Anth (Chlorpyriphos + Cypermethrin) on seed germination and seedling growth of *Glycine max* L.Merr.

1. Control, 2. 0.25 %, 3. 0.5 %, 4. 1 %, 5. 2 %

PLATE 4





Influence of 12 h seed treatment of Anth (Chlorpyriphos + Cypermethrin) on seed germination and seedling growth of *Glycine max* (L.) Merr.

1. Control, 2. 0.25 %, 3. 0.5 %, 4. 1 %, 5. 2 %

(Chlorpyriphos and Cypermethrin) at both the exposure periods caused increase in fresh weight and dry weight over control. The level of phytotoxicity caused detrimental effect at higher concentration with 12 h exposure period by the Anth (Chlorpyriphos and Cypermethrin) .

Table – 6 : Effect of Anth (Chloropyriphos and Cypermethrin) on seedling growth of *Glycine max* (L.) Merrill

Exposure Period	Concentrations (%v/v)	RL (cm)	SL (cm)	R/S	RLN	FW (g)	DW (g)	PP
6h	Control	4.96 ±2.43	4.67 ±1.14	1.06	5.84 ±1.94	0.41	0.12	-
	0.25	4.89 ±1.69	3.15 ±0.90	1.54	8.0 ±3.57	0.69	0.15	1.41
	0.5	5.92 ±1.70	4.7 ±1.56	1.25	6.67 ±1.87	0.44	0.11	-19.35
	1.0	4.52 ±1.21	4.1 ±0.21	1.20	6.34 ±1.75	0.41	0.10	0.80
	2.0	4.27 ±1.90	3.42 ±0.77	1.25	6.34 ±1.21	0.38	0.10	13.91
12h	Control	5.53 ±1.73	2.7 ±0.60	2.04	4.81 ±2.23	0.40	0.12	
	0.25	4.22 ±1.40	2.6 ±1.06	1.62	8.71 ±2.20	0.46	0.11	23.68
	0.5	7.97 ±1.32	5.33 ±0.70	1.48	10.6 ±3.17	0.56	0.11	-43.4
	1.0	6.97 ±2.58	1.69 ±0.51	4.12	10.37 ±1.69	0.42	0.11	-26.03
	2.0	3.89 ±1.88	1.55 ±0.54	2.50	2.0 ±1.61	0.42	0.09	29.65

SL- Shoot Length
RL- Root Length
FW-Fresh Weight
DW- Dry Weight

R/S- Root to shoot ratio
RLN-Rootlet Number
PP - Percent Phytotoxicity

Values are mean of ± S.D. based on three replicates.

An adverse effect of insecticide treatment on shoot and root development has been reported in several studies. With increasing concentrations of monocrotophos, a retardation in the root and shoot length and number of laterals and nodules was clearly observed in *Trigonella* (Kamble and Sabale 2001). The seed treatment with aldrin, monocrotophos, dimecron, endosulfan, dematon and carbofuran caused harmful effect on germination and growth in jowar, groundnut, okra and *Trigonella* seeds (Benjamini,1986 ; Gupta et al.1983 ;Agarwal and Bhanot, 2000). Sengupta et al. (1986) reported that malathion has adverse effect on wheat germination, root and shoot growth. Utpalnath and Jayapragasam (1993) studied effect of different insecticides on growth of rice variety IR 50. They observed that sumicidin treatment had promoted hypocotyl length slightly but had negative effect on root length, They also observed that nuvan had adverse effect on root and hypocotyl length. Sabale and Misal (1993) observed negative effect of methyl parathion on root and shoot development of Jowar. Patil (1995) observed that root and shoot length was hampered with increasing concentration of monocrotophos and monocrotophos in combination with bavistin in soybean. Thus mostly a negative influence of insecticides on seedling growth has been reported on most of the crops. Our observations also reveal similar trend in *Glycine max* L. Merr. and observed that Anth (Chlorpyriphos and Cypermethrin) treatment caused inhibitory effect on seedling growth of *Glycine max*(L.)Merr.

3.Organic Constituents :

Pesticides influence various physiological and biochemical processes, which may result in a beneficial effect on the crops by acting as a tonic or stimulant to the growth of plants (Vyas 1993 ; Vyas and Nene 1974). As pesticides are chemicals, they act to control diseases in many ways. When we use chemicals to inhibit or kill fungi, it is possible that these chemicals affect plants and enhance its nutritional value. While entering the plant and afterwards they encounter a number of physiological and biochemical processes, some compounds are converted into fungitoxic products while a few become innocuous and still others modify the host physiology in such a way that plant acquires resistance against diseases.

Seed germination and growth analysis of *Glycine max*(L.)Merr. was carried exposing seeds to 6 and 12 h exposure periods and a wide range of concentrations of Mancozeb and Anth (Chlorpyriphos + Cypermethrin). From these studies a few concentrations were selected for further study in order to analyse their effects on a few physiological and biochemical parameters in the seedlings.

a. Carbohydrate content :

Influence of Mancozeb on carbohydrate content in germinating seeds of *Glycine max*(L.)Merr. is depicted in Table 7 and fig. 3 and 4.

Table 7 : Effect of Mancozeb on carbohydrate content in germinating seeds of *Glycine max*(L.)Merr.

Exposure Period	Pesticide	Reducing sugars	Total sugars	Starch	Total carbohydrates
6 h	Control	0.19	0.38	2.27	2.65
	Mancozeb (% w/v) 0.25	0.06	0.29	1.00	1.27
	0.5	0.06	0.22	0.64	0.86
	1.0	0.2	0.2	1.05	1.25
	2.0	0.15	0.34	1.86	2.20
12 h	Control	0.13	0.22	0.2	0.42
	Mancozeb (% w/v) 0.25	0.13	0.18	0.17	0.35
	0.5	0.18	0.24	0.25	0.49
	1.0	0.09	0.36	0.21	0.57
	2.0	0.07	0.36	0.16	0.52

Values are expressed in g / 100 g fresh weight

It can be seen from the table that fungicide Mancozeb significantly caused reduction in total sugars and starch content at 6 h exposure period. Stimulation in total sugars and starch content was observed at 0.5 % and 1 % concentrations at 12 h exposure period . Decreased reducing sugars and starch content was observed at higher and lower concentrations viz. 0.25 % and 2 %. All the concentrations of Mancozeb at both exposure periods showed decrease in reducing sugars of soybean. A slight increase in the content of reducing sugars was observed by 1 % and 0.5 % concentrations of Mancozeb at 6 and 12 h period . Decreased reducing sugars and starch content was observed at higher and lower concentrations viz. 0.25 % and 2 %. All the concentrations of Mancozeb at both exposure periods showed decrease in reducing sugars of soybean. A slight increase in the content of reducing sugars was observed by 1 % and 0.5 % concentrations of Mancozeb at 6 and 12 h exposure periods respectively.

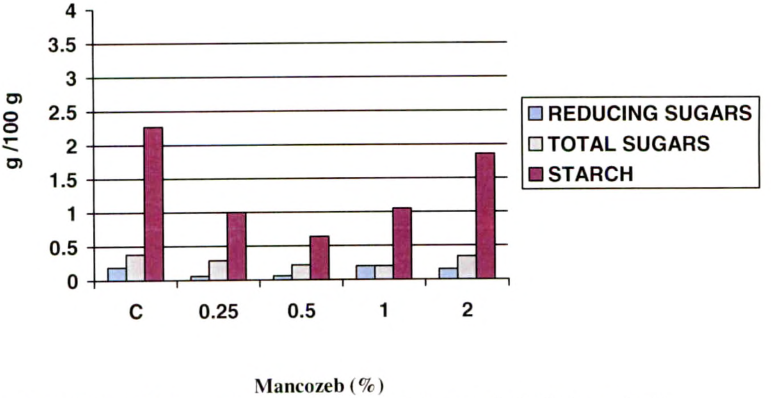
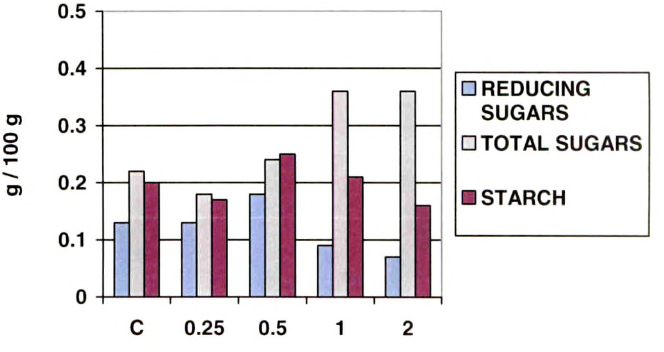


Fig 3: Effect of 6 h seed treatment of Mancozeb on carbohydrate content in germinating seeds of *Glycine max*(L.)Merr.



Mancozeb (%)

Fig . 4 : Effect of 12 h seed treatment of Mancozeb on carbohydrate content in germinating seeds of *Glycine max*(L.)Merr.

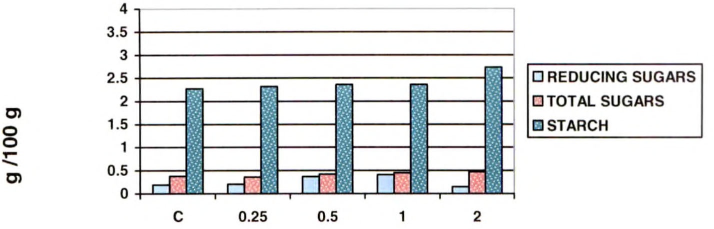
Effect of insecticide Anth (Chlorpyriphos + Cypermethrin) on carbohydrate content in germinating seeds of *Glycine max*(L.)Merr. is depicted in Table 8 and fig. 5 and 6.

Table 8 : Effect of Anth (Chlorpyriphos + cypermethrin) on carbohydrate content in germinating seeds of *Glycine max*(L.)Merr.

Exposure period	Pesticide	Reducing sugars	Total sugars	Starch	Total Carbohydrates
6 h	Control	0.19	0.38	2.27	2.65
	Anth(Chlorpyriphos +Cypermethrin) (% v/v)				
	0.25	0.21	0.36	2.32	2.68
	0.5	0.37	0.42	2.36	2.78
	1.0	0.41	0.45	2.36	2.81
	2.0	0.15	0.47	2.73	3.2
12 h	Control	0.13	0.22	2.00	2.22
	Anth(Chlorpyriphos +Cypermethrin) (% v/v)				
	0.25	0.24	0.27	2.27	2.54
	0.5	0.29	0.32	2.45	2.77
	1.0	0.22	0.33	2.86	3.19
	2.0	0.13	0.36	2.91	3.27

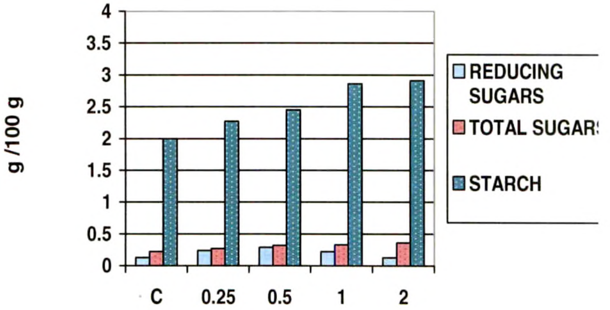
All values are expressed in g / 100 g fresh weight .

It can be seen from the table that total sugars and starch content was found increased with increase in concentration of Anth (Chlorpyriphos + Cypermethrin) at both the exposure periods. Slight increase was observed in total sugars and starch at 6 h exposure treatment. Increase in reducing sugars was observed with increase in concentration of Anth (Chlorpyriphos + Cypermethrin) at both the exposure periods.



Anth (Chlorpyriphos + Cypermethrin) (%)

Fig. 5 : Effect of 6 h seed treatment of Anth (Chlorpyriphos + Cypermethrin) on carbohydrate content in germinating seeds of *Glycine max* (L.) Merr.



Anth (Chlorpyriphos + Cypermethrin) (%)

Fig. 6 : Effect of 12 h seed treatment of Anth (Chlorpyriphos + Cypermethrin) on carbohydrate content in germinating seeds of *Glycine max* (L.) Merr.

But at higher concentration viz. 2 % of insecticide slight decrease was observed. Concentrationwise increase in total carbohydrate content was observed with increase in concentration of pesticide with both the exposure periods.

Positive and negative effects of pesticides on carbohydrate have been reported in several studies. Sugavanam (1992) observed stimulation in soluble sugars in bengal gram (*Cicer arietinum*) with lower concentration of Mancozeb. Carbendazim treatment stimulated the level of total sugars of *Quercus serrata* (Ghosh and Srivastava 1994). Kalebere and Sabale (2000) observed increase in total sugars and starch content in onion with the application of carbendazim. Carbendazim application stimulated the carbohydrate content while monocrotophos treatment declined it in *Trigonella* (Kamble and Sabale 2002). Sharma and Thakore (2004) reported increase in starch content in chilli with the application of systemic fungicides.

Prasad and Mathur (1983) observed increase in reducing sugars in *Vigna mungo* L. Hepper with the treatment of metasystox. Santhguru (1990) observed decline in soluble sugars in *Cyamposis tetragonoloba* TAUB with increasing concentration of rogor. Sundarraj et al.(1993) studied effect of insecticides viz. cypermethrin, monocrotophos, combination of chlorpyriphos and cypermethrin (1 : 10) on biochemical parameters of cotton and reported that these insecticides did not significantly influence reducing sugars, non reducing sugars and total sugars. Sarasvati et al. (1996) reported that phorate and sumicidin treatment affect the level of reducing sugars in soybean. Our observations also reveal similar trend in *Glycine max* L. Merr.

b. Total soluble proteins :

The protein content in germinating seeds of *Glycine max*(L)Merr. treated with Mancozeb and Anth (Chlorpyriphos + Cypermethrin) are recorded in table 9. It can be seen from the table that seed treatment with 6 h exposure period with Mancozeb caused increase in protein content over control. In 12 h exposure treatment seed treatment, the protein level increased with the treatment of 2 % Mancozeb, lower concentrations upto 0.5 % significantly caused reduction in protein level. As per increase in concentrations of Anth (Chlorpyriphos + Cypermethrin) seed treatment with both exposure periods, accumulation of proteins in germinating was recorded.

Sugavanam (1992) reported that lower concentration of Mancozeb increased the protein content while higher concentration decreased it. Percentage of protein increased as as concentration of pesticide was increased and the treatment of bavistin was found more stimulatory than that of monocrotophos (Sabale and Kamble 1995). Methyl parathion treatment has been reported to increase protein level in seeds of bengal gram (Sengupta et al. 1986 ; Thirumaran and Xavier 1987). Pathak and Mukherji (1986) reported stimulation in protein content by insecticide sevin. Mathur et al. (1989) have reported that phorate treatment increased protein content in *Vigna mungo* L. Sukul and Handa (1989) observed that deltamethrin, cypermethrin and permethrin did not cause any adverse effect on protein content in bengal gram.

Table 9 : Effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) on Total soluble proteins and Total polyphenols in germinating seeds of *Glycine max*(L.)Merr.

Exposure Period	6 h		12 h	
Pesticide	Total soluble proteins	Total polyphenols	Total soluble proteins	Total polyphenols
Control	11	0.20	12	0.17
Mancozeb (% w/v)				
0.25	11	0.20	10	0.20
0.5	11	0.20	11	0.22
1.0	12	0.25	12	0.25
2.0	13	0.31	13	0.30
Control	11	0.20	12	0.17
Anth(Chlorpyriphos +Cypermethrin) (% v/v)				
0.25	11	0.21	12	0.25
0.5	13	0.25	13	0.21
1.0	15	0.30	18	0.20
2.0	19	0.31	18	0.18

Values are expressed in g / 100 g fresh weight .

Patil (1997) reported that lower concentration of monocrotophos increased protein content while higher concentration was inhibitory. Pratibha and Gupta (2006) reported that fungicides dithane, ridomil and insecticide endosulfan increased total soluble proteins in maize.

Sukul and Handa (19890 reported adverse effect of pesticides on protein content of *Trigonella*. Our findings in *Glycine max* L.Merr. also reveal the similar trend to that other pesticide treatments viz. bavistin, methyl parathion, sevin etc.

c. Total polyphenol content :

It can be seen from the table 9 that Total polyphenols increased in *Glycine max* L. Merr. with increase in concentration of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment at both exposure periods. Treatment of higher concentration of both the pesticides have stimulated the level of polyphenols in *Glycine max* (L.) Merr.

Thirumaran and Xavier (1987) also reported increase in total polyphenols in *Vigna mungo* L. with methyl parathion treatment. Kamble and Sabale (2002) reported similar observations i.e. stimulation in polyphenol content in *Trigonella* with the treatment Carbendazim and monocrotophos.

Polyphenols are the secondary metabolites. Polyphenols are the aromatic compounds formed during secondary metabolism and play an important role in disease resistance (Wallace and Mansell, 1975). Secondary products present in plants are believed to play important role in disease resistance, pathogenic attack or any type of stress (Harborne, 1986). In oxidation - reduction reactions, phenolic compounds function as donors or acceptors of hydrogen ions. Phenolics also interfere with growth and other energy dependant activities by uncoupling oxidative phosphorylation. The phenolic compounds in some instances affect fundamental processes such as photosynthesis, plant water relations, protein synthesis and membrane permeability (Rice, 1979 ; Glass and Dunlop, 1974).

4. Enzyme studies :

While entering the plant and afterwards pesticides encounter a number of physiological and biochemical processes which are mediated by several enzymes that are often altered by these pesticides. Enzymes are the biocatalysts which regulate various biochemical reactions taking place in cells. Activities of different enzymes get altered when plants are exposed to pesticides.

a. Protease [EC 3.4.3.2] :

Effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment on the activity of enzyme protease in germinating seeds of *Glycine max* (L.) Merr. was investigated and recorded in Table 10 .

Table 10 : Effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) on the activity of Protease and Nitrate Reductase in germinating seeds of *Glycine max* (L.)Merr.

Exposure period Pesticide	6 h		12 h	
	Protease *	Nitrate Reductase @	Protease *	Nitrate Reductase @
Control	9.13	0.3	9.84	0.9
Mancozeb (% w/v)				
0.25	9.13	1.2	8.41	0.72
0.5	8.41	0.96	8.41	0.6
1.0	6.99	0.9	7.14	0.48
2.0	6.28	0.6	6.99	0.42
Anth(Chlorpyriphos +Cypermethrin) (% v/v)				
0.25	8.41	0.6	8.69	0.6
0.5	6.28	1.2	6.99	1.8
1.0	5.57	3.0	5.56	2.4
2.0	6.99	4.8	5.28	4.2

* Values are expressed in µg tyrosine liberated h^{-1} g^{-1} fresh weight.
@ Values are expressed in µg NO_2 produced h^{-1} g^{-1} fresh weight .

It is evident from the table that protease activity is inhibited with increase in concentration of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) with both the exposure periods i.e. 6 h and 12 h. The decline in enzyme activity was remarkable at highest concentration of both the pesticides especially at 12 h exposure period.

Pathak and Mukherji (1986) reported stimulation in protease activity by sevin. Sabale and Misal (2000) found an increase in protease activity at lower dose of endosulfan and malathion. Sengupta et al. (1986) found significant inhibition in protease activity due to carbaryl, malathion and BHC treatments in wheat and *Vigna sinesis*. Kamble and Sabale (2002) found an inhibitory effect of monocrotophos on protease activity in *Trigonella*. Our observations also reveals similar trend in *Glycine max* L. Merr. These results in general indicate that pesticidal stress inhibited protein breakdown which results in lowering the enzyme level.

b . Nitrate Reductase [EC 1.6.6.1] :

The activity of Nitrate Reductase in response to Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment in *Glycine max* (L.) Merr. germinating seeds is depicted in table 10. It is evident from the table that positive and negative effect of Mancozeb seed treatment was observed on Nitrate Reductase in seeds of *Glycine max* L. Merr. treated at 6 h and 12 h exposure period respectively. The activity of Nitrate Reductase was positively influenced in the germinating seeds treated with Mancozeb with 6 h exposure period. Maximum activity was recorded for 0.25 % Mancozeb, showing more than three fold increase in the enzyme level. The enzyme activity decreased with increasing concentration of Mancozeb. However even at maximum dose of Mancozeb, the enzyme level was not less than that of untreated seeds. The response obtained with 12 h exposure period seed treatment with Mancozeb was exactly opposite showing steady decrease in enzyme activity with increased concentration of Mancozeb. At maximum dose of Mancozeb, the enzyme level dropped down to about one third of the control seeds. The activity of Nitrate Reductase was positively influenced in the germinating seeds treated with Anth (Chlorpyriphos + Cypermethrin) at both the exposure periods. In germinating seeds treated with Anth (Chlorpyriphos + Cypermethrin) at both the exposure periods i.e. 6 h and 12 h all the concentrations of

Anth (Chlorpyriphos + Cypermethrin) increased Nitrate Reductase activity with increase in concentration of pesticide. At maximum concentration of Anth (Chlorpyriphos + Cypermethrin), maximum activity of enzyme was observed.

Kamble and Sabale (2002) reported positive influence of carbendazim and monocrotophos on seed treatment in *Trigonella*. Patil et al. (1997) reported inhibition in Nitrate Reductase activity in soybean by higher concentrations of monocrotophos.

Nitrate Reductase is an important enzyme of nitrogen metabolism which catalyses the first step of nitrogen assimilation. It brings about reduction of nitrate to nitrite. During the reduction, electrons are directly transferred from molybdenum to nitrite (Gurrero et al. 1981).Nitrate Reductase activity is dependant upon the availability of Nitrate and NADH. The enzyme is assayed using an in vivo method where nitrate is supplied from outside.

Our results with positive and negative effect on the activity of Nitrate Reductase enzyme of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment reveals the susceptibility of nitrogen metabolism with pesticide treatment during seed germination.

c. Acid phosphatase [EC 3.1.3.2]:

Effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment on the activity of enzyme acid phosphatase activity in seeds of *Glycine max*(L.) Merr. is represented in table 11. It is evident from the table that At both the exposure periods, seed treatment with Mancozeb concentration wise reduction in acid phosphatase activity. At the highest concentration of Mancozeb viz. 2 % , at 12 h exposure period the enzyme activity dropped down to about half of the control seeds.

On the other hand, seed treatment of Anth (Chlorpyriphos + Cypermethrin) with both the exposure periods caused stimulation in enzyme activity upto 1 % concentration. Only higher concentration viz. 2 % caused reduction in enzyme activity over control.

Sengupta et al. (1986) and Soam (1990) observed positive and negative influence on acid phosphatase activity on exposure to insecticides. Acid phosphatase activity was found stimulated at higher doses of bavistin but was supressed by the monocrotophos treatment (Kamble and Sabale, 2002).

Table 11 : Effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) on the activity of Acid Phosphatase and Catalase in germinating seeds of *Glycine max*(L.)Merr.

Exposure period	6 h		12 h	
Pesticide	Acid Phosphatase*	Catalase@	Acid Phosphatase*	Catalase@
Control	19.6	1.08	44.0	1.4
Mancozeb (% w/v)				
0.25	18.4	1.08	40.8	1.6
0.5	20.6	1.08	32.4	1.4
1.0	18.2	1.9	30.8	1.4
2.0	14.4	2.1	22.0	1.3
Anth (Chlorpyriphos+ Cypermethrin) (% v/v)				
0.25	47.2	1.2	50.0	1.9
0.5	46.4	1.3	48.0	1.9
1.0	38.4	1.3	45.2	1.1
2.0	13.6	1.4	42.4	0.6

* Values are expressed in Δ OD /h/g fresh weight.
@ Values are expressed in mg H_2O_2 broken down/ min/g fresh tissue.

Acid phosphatase is a hydrolytic enzyme which brings about breakdown of several sugar phosphates and ATP (DeLeo and Sacher, 1970). When plants are treated with pesticides, it is quite obvious that pesticidal stress causes an increase in the level of hydrolytic enzymes as found in the present study with lower concentrations of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) at 6 h exposure period. In the Mancozeb treatment at 12 h exposure period, an inhibition of enzyme activity was noticed in the present study, suggesting decreased level of hydrolysis during germination.

d. Catalase [EC 1.11.1.6] :

The effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) on the activity of catalase in germinating seeds of *Glycine max*(L.)Merr. is depicted in table 11.

It is evident from the table that seed treatment of Mancozeb with 6 h exposure period marginally stimulated catalase activity with increase in concentration.While seed treatment of Mancozeb with 12 h exposure period did not cause marked increase or decrease in catalase activity.

Seed treatment of Anth (Chlorpyriphos + Cypermethrin) with 6 h exposure period caused marginal stimulation in catalase activity with increase in concentration of Anth. In seeds treated with Anth (Chlorpyriphos + Cypermethrin) at 12 h exposure period, lower concentration positively influenced catalase activity. Maximum concentration of Anth caused reduction in catalase activity over control.

Reddy and Vidyavati (1983) have reported an increase in catalase activity in *Dolichos biflorus* by kitazin seed treatment. Catalase is generally involved in regulating H_2O_2 level in cells which otherwise would be toxic to the cell. A remarkable increase in catalase activity with pesticide treatment during seed germination in *Glycine max* L. Merr.reveals to protect seeds from accumulation of H_2O_2 during germination.

5. Mineral Analysis :

Inorganic elements such as Calcium, Magnesium, Manganese, iron, zinc and copper were estimated from the acid digest of oven dried plant material after pesticide treatment. Pesticide treatment at 6 h and 12 h exposure periods to germinating seeds has been recorded in Table 12 and 13.

Calcium :

Content of Calcium in *Glycine max*(L.) Merr. seeds treated with Mancozeb and Anth (Chlorpyriphos + Cypermethrin) at 6 h and 12 h exposure periods is depicted in Table 12. It is evident from the table that at 6 h exposure period seed treatment with Mancozeb, lowest concentration viz. 0.25 % caused decline in Ca content. Higher

Table 12 : Effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment on Major elements of *Glycine max* (L.) Merr.

Exposure period	6 h			12 h		
Pesticide	Fe	Ca	Mg	Fe	Ca	Mg
Control	0.5	3.45	0.57	0.4	3.58	0.53
Mancozeb (%w/v) 0.25	0.2	2.97	0.45	0.3	4.07	0.84
0.5	0.3	3.46	0.55	0.3	4.43	0.80
1.0	0.3	3.65	0.59	0.3	4.5	0.78
2.0	0.3	3.90	0.63	0.3	4.62	0.74
Anth (Chlorpyriphos + Cypermethrin) (%v/v) 0.25	0.5	2.21	0.54	0.4	2.98	0.54
0.5	0.3	3.35	0.45	0.4	3.5	0.55
1.0	0.3	3.45	0.43	0.4	3.76	0.57
2.0	0.3	3.52	0.40	0.5	4.24	0.63

Values are expressed in g/ 100 g dry weight.

concentrations caused accumulation in Ca content with increase in concentration of Mancozeb. At 12 h exposure period seed treatment with Mancozeb, Ca level increased steadily with increase in concentration of Mancozeb.

Seed treatment of Anth (Chlorpyriphos + Cypermethrin) with both the exposure periods viz. 6 h and 12 h caused decline in Ca content at lowest concentration, while other concentrations caused accumulation in Ca content with increase in concentration of Anth (Chlorpyriphos + Cypermethrin).

Calcium functions both as structural component as well as cofactor for certain enzymes. Calcium plays an important role in regulation of membrane permeability of various ions, in particular inorganic cations (Vansteveninck, 1965). According to Clarkson and Hanson (1980) Calcium binds with proteins, nucleic acids and lipids and affects cell adhesion, membrane chromatin organization and enzyme conformation. Calcium stimulates or inhibits enzymes like ATPase, protein kinase, nucleases, pectin esterases, lipoxygenase, alpha amylase, pyruvate kinase, polygalacturonic transleminase (Clark, 1984). According to Hanson Calcium minimizes ion diffusion, maintains selective ion transport mechanism and decreases membrane permeability. Calcium enhances the net absorption of potassium (Ortiz et al. 1994). Evans et al.(1991) recognised Calcium as a transducer of hormonal and environmental signals to the responsive elements of cell metabolism. Marme (1989) reported that Calcium affects a large number of biochemical reactions. Jones et al. (1993) reported that Calcium activates and stabilizes the alpha amylase molecule. A plant deficient in it shows stem collapse and subsequent termination of growth in apical region.(Chapman, 1966). Calcium is required for cell elongation and cell divisions (Burstorm, 1968). Calcium might influence growth directly or indirectly through cell divisions, middle lamellar deposition, ion regulation, related osmotic responses and cell wall structure (Rains 1972). Calcium protects plant from the injurious effects of H^+ ions (Rains et al. 1964), high salts in the environment (Rains, 1972) and other potentially toxic ions present in the environment (Arnold, 1969). In *Glycine max* L. Merr., Ca content is declined at low concentrations and increased at higher concentrations. This may be considered as an adaptive feature to create resistance mechanism in this plant with pesticide treatment.

Magnesium :

It is evident from the table 12 that at 6 h exposure period seed treatment, lower concentrations of Mancozeb caused decline in Mg content. While higher concentrations caused increase in Mg content in treated seeds over control. At 12 h exposure period seed treatment, all concentrations of Mancozeb increased Mg content in treated seeds of *Glycine max*(L.)Merr.

Anth (Chlorpyriphos + Cypermethrin) seed treatment with 6 h exposure period caused steady decline in Mg content with increase in concentration of Anth

(Chlorpyriphos + Cypermethrin). Exactly reverse results are obtained with 12 h seed treatment with Anth (Chlorpyriphos + Cypermethrin). Anth (Chlorpyriphos + Cypermethrin) seed treatment with 12 h positively influenced Mg level in treated seeds.

Magnesium is relatively a mobile element and is found both in bound as well as free form (Gilbert, 1957). It serves as structural component of cells. It is also involved in structure of chlorophylls and requirement of it in chlorophyll was originally inferred from the observations of chlorosis when Magnesium was limiting . Head region of chlorophyll is made up of porphyrin ring with Mg^{++} in centre. Magnesium stabilizes the ribosomal particles in the configuration necessary for protein synthesis (Mengel and Kirkby,1982). Magnesium also plays very significant role in organic acid metabolism in plants. Magnesium is highly essential for many enzymatic reactions as it acts as a cofactor in almost all enzymes activating phosphorylation processes (Clark, 1984). Magnesium is often associated with organic anions such as malate, citrate, pectate as well as inorganic ions (Kirkby and Mengel, 1967). Moore et al. (1961) have reported that Magnesium is essential for malic and citric acid synthesis. It is commonly associated with transfer reactions involving phosphate reductive groups (Nason and McElory, 1963).Almost every phosphorylating enzyme in carbohydrate metabolism requires Magnesium for maximal activity. Hatch et al. (1974) and Mukherji (1974) have shown that Magnesium is involved in the mechanism of carboxylation reactions. It is required in the activity of malate dehydrogenase and malic enzyme (Hatch et al. 1975).

Present findings with *Glycine max*(L.) Merr. indicate that Mg content in treated seeds increases with increase in concentration of both the pesticides. This may be an adaptive feature to retention of chlorophylls.

Iron :

It can be seen from the table 12 that seed treatment of Mancozeb with both the exposure periods negatively influenced Fe content in treated seeds. Seed treatment of Anth (Chlorpyriphos + Cypermethrin) with 6 h exposure period also negatively influenced Fe content. Decrease in Fe content was observed with respect to the concentration of Anth (Chlorpyriphos + Cypermethrin). Seed treatment of Anth (Chlorpyriphos + Cypermethrin) with 12 h exposure period caused slight increase in Fe with an increase in concentration. Maximum content of Fe was observed at highest concentration of Anth (Chlorpyriphos + Cypermethrin).

Like other elements, Iron functions both as structural component as well as a cofactor for enzymatic reactions. Iron exists in plants in the form of haemproteins like cytochromes and iron-sulfur proteins (Sandman and Boger, 1983). Cytochromes are involved in electron transport system in chloroplast and mitochondria and enzymes viz. catalase, cytochrome oxidase. In nonhaem iron-sulfur proteins, the iron is coordinated to the thiol group of cysteine and/ or to inorganic sulphur. The most important is ferredoxin which act as an electron transmitter in a number of metabolic reactions of photosynthesis, nitrite reductase. Iron deficiency markedly reduces Nitrate Reductase activity and haemoglobine concentration in soybean nodules (Cheniae and Evans, 1960). In plants a large portion of Iron is associated with porphyrins. Fe is essential in formation of protochloro-phyllide from Magnesium- protoporphyrin during biosynthesis of chlorophylls. Various investigations have estimated that as much as 75 % total cell iron is associated with the chloroplasts. It has been estimated that Iron is a constituent of peroxidase, catalase and cytochrome oxidase.

Our results with Mancozeb and Anth (Chlorpyriphos + Cypermethrin) indicate that in seeds of *Glycine max* (L.) Merr. there may be positive or negative effects with both the pesticides on Fe content.

Manganese :

It can be seen from the table 13 that seed treatment with Mancozeb at both the exposure periods increased Manganese content in treated seeds with increase in concentration. Seed treatment of Anth (Chlorpyriphos + Cypermethrin) with both the exposure periods viz. 6 h and 12 h caused decrease in Mn content in treated germinating seeds.

Manganese is associated most commonly with its role in photosynthesis. Manganese is essential in all plants for 'Hill reaction' in the water splitting and O_2 evolving system in photosynthesis. PS-II contains manganoprotein which catalyses early stage of O_2 evolution. It is also involved in oxidation-reduction processes, decarboxylation and hydrolysis reactions. It can replace Mg in many of the phosphorylating and group transfer reactions. Manganese brings about the oxidation of IAA oxidase (Mumford et al. 1962).In majority of the enzyme systems, Manganese is as effective as Mg in promoting enzyme activities. Manganese is also required for maintainance of chloroplast (Teichler-Zallen, 1969). Manganese plays an important role in many enzyme systems. It plays an important role in maintaining the auxin level inside the plant body through the enzyme system IAA-oxidase. It is directly involved as a component of biotin enzyme in the biosynthesis of fatty acids (Marschner, 1986).

Table 13 : Effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment on Micronutrient level of *Glycine max* (L.) Merr.

Exposure period	6 h			12 h		
Pesticide	Cu	Zn	Mn	Cu	Zn	Mn
Control	5.68	28.5	9.4	4.26	24.4	8.4
Mancozeb (%w/v)						
0.25	4.44	24.0	22.2	4.28	39.2	23.2
0.5	4.56	31.6	31.0	4.30	34.8	66.8
1.0	4.8	32.0	35.6	4.34	34.4	70.0
2.0	5.08	32.8	40.2	4.44	34.4	75.6
Anth(Chlorpyriphos + Cypermethrin) (%v/v)						
0.25	7.12	38.12	7.6	5.18	38.16	7.4
0.5	4.58	22.0	8.4	5.04	38.8	7.8
1.0	4.00	21.2	8.6	5.00	39.2	8.0
2.0	3.8	20.84	8.8	3.76	34.44	8.2

Values are expressed in mg/ 100g dry weight.

Zinc :

It is evident from the table 13 that at 6 h exposure period lowest concentration viz.

0.25 % of Mancozeb caused decrease in Zn content in treated seeds. Higher concentrations of this pesticide caused accumulation of zinc content in treated seeds. Seed treatment of Mancozeb with 12 h exposure period caused accumulation of zinc content in seeds. Highest content of zinc was observed at lowest concentration of Mancozeb. At 6 h exposure period seed treatment, lowest concentration of Anth (Chlorpyriphos + Cypermethrin) caused slight increase in Zn content in treated seeds. While other concentrations decreased it in treated seeds. All concentrations of Anth (Chlorpyriphos + Cypermethrin) at 12 h exposure period seed treatment caused accumulation of zinc content than untreated seeds.

Zinc is one of the important trace elements in plants. It acts either as a metal component of enzymes or as a structural, functional and regulatory factors of a large number of enzymes. There are some zinc-metallo enzymes present in plants like glutamic acid dehydrogenase, lactic acid dehydrogenase, alcohol dehydrogenase, proteinases and peptidases.

Copper :

It is evident from the table 13 that seed treatment of Mancozeb with 6 h exposure period caused decline in copper level in treated germinating seeds, while12 h exposure period seed treatment caused accumulation of copper content in treated seeds. A slight increase in copper content was observed in seeds treated with Mancozeb at 12 h exposure period. Only lowest concentration of Anth (Chlorpyriphos + Cypermethrin) at 6 h seed treatment caused accumulation of Cu content, while other concentrations of Anth (Chlorpyriphos + Cypermethrin) caused steady decrease in copper level. Seed treatment of Anth (Chlorpyriphos + Cypermethrin) with 12 h exposure period caused increase in copper content.

Copper plays an important role in maintaining membrane structure of thylakoids (Henriques, 1989). It is an essential component of cytochrome oxidase complex of mitochondrial electron transport chain. Enzymes like superoxide dismustase, ascorbate oxidase, amino oxidase requires copper. Copper is an effective inhibitor of vegetative growth and induces general symptoms of senescence.

B. INFLUENCE OF SEED TREATMENT AND FOLIAR SPRAY :

1. Seedling Growth :

The effect of seed treatment and foliar spray of Mandy M -45 (Mancozeb 75 %W.P.) and Anth (Chlorpyriphos 50 % and Cypermethrin 5%) was studied on growth of *Glycine max* (L.) Merr.

a. Effect of Mandy M -45 (Mancozeb 75 % W.P.) :

i. Shoot Growth :

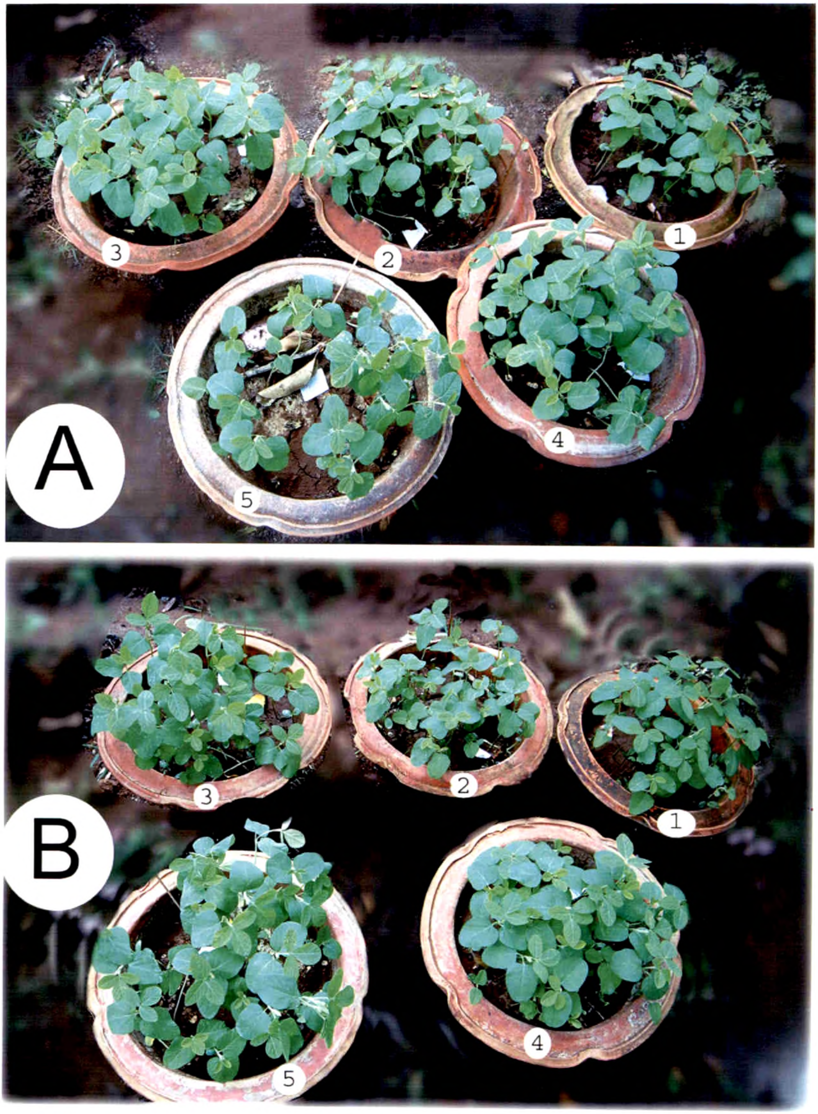
The influence of Mancozeb seed treatment at 6 and 12 hour exposure periods and foliar spray on shoot length of *Glycine max* (L.) Merr. is depicted in Table 14. It is evident from the table that both the 6 h and 12 h exposure period seed treatments and foliar spray of Mancozeb caused stimulation in shoot length.

Many workers have reported a positive effect of fungicides on growth in different plants. Sundarvelu et al. (1998) reported stimulation in height of okra following seed treatment and foliar spray of dithane and thirum. Foliar sprays of benzimidazole at weekly intervals increased plant height in *Phyllanthus urinaria* (Dogra et al.1986). Narsimhudu and Balsubramanian (2001) have been observed influence of Carbendazim treatment on the height of turmeric. Similar results were reported by Shivankar et al. (2000) on the growth of wheat by Carendazim treatment. Kamble and Sabale (2002) observed stimulation in overall growth of fenugreek. They noticed increase in average shoot length by all concentrations of Bavistin. Dhopte et al. (1995) observed reduction in height of peanut by paclobutrazol spray. Kannayian and Prasad (1982) noticed that Benlate, deconil, planavax and vitavax seed treatment caused significant increase in shoot length in rice. Triadimenole and triticonazole, applied as seed treatment affected plant growth, shoot development and root axis production (Montfort 1996). Buta (1991) noticed reduction in height of wheat seedlings by paclobutrazol treatment.

ii. Root Growth :

The seed treatment of Mancozeb with 6 h exposure period and foliar spray caused increase in root length with all the concentrations of Mancozeb. While at 12 hr exposure period, seed treatment with highest concentration of Mancozeb caused decrease in root length while other concentrations were positively influenced.

PLATE 5



Influence of seed treatment and foliar spray of Mancozeb on growth of *Glycine max* (L.) Merr.

1. Control , 2. 0.25%, 3. 0.5%, 4. 1 %, 5. 2%

A . 6 h seed treatment B. 12 h seed treatment

Table 14 : Effect of Mancozeb seed treatment and foliar spray on growth of *Glycine max*(L.) Merr.

Exposure Period	Pesticide	R L (cm)	S L (cm)	N D	L	F W (g)	D W (g)
6 h	Control	11.0 (±4.06)	15.9 (±2.41)	7 (±3.65)	5 (±1.41)	1.42	0.15
	Mancozeb (%w/v)						
	0.25	11.6 (±0.89)	22.0 (±1.58)	8 (±0.1)	6 (±1.64)	1.48	0.15
	0.5	12.4 (±1.52)	21.5 (±0.86)	8 (±0.1)	8 (±0.1)	1.55	0.16
	1	13.8 (±2.68)	19.2 (±2.59)	6 (±2.54)	7 (±1.64)	1.62	0.17
	2	11.8 (±1,64)	18.4 (±3.81)	5 (±2.32)	6 (±1.64)	1.46	0.15
12 h	Control	11.0 (±1.22)	15.2 (±1.22)	11 (±4.28)	7 (±1.34)	1.70	0.2
	Mancozeb (%w/v)						
	0.25	14.4 (±2.07)	17.2 (±1.30)	8 (±3.03)	7 (±1.34)	1.48	0.15
	0.5	14.6 (±0.89)	22.0 (±1.58)	8 (±3.03)	8 (±0.1)	1.58	0.16
	1	14.8 (±1.61)	22.5 (±1.41)	3 (±1.26)	8 (±0.1)	1.58	0.16
	2	10.4 (±1.14)	20.8 (±5.1)	2 (±2.2)	7 (±1.34)	1.56	0.15

SL : Shoot length
RL : Root length
ND : Nodule number per plant
(Values are mean of three replicates.)

FW : Fresh weight per plant
DW : Dry weight per plant
L : Number of leaves per plant

Kamble and Sabale (2002) also reported stimulation in root growth by Carbendazim treatment in *Trigonella.* Somashaker and Sreenath (1987) observed reduction in root length with Benalate application in *Crotalaria juncia*. Reis and Melo (1982) noticed decreased number of roots in wheat with Benomyl. On the contrary in the present study lower concentrations of Mancozeb treatment stimulated root growth over control. The lower concentration stimulate the root length more than the higher concentrations upto 1%, the highest concentration caused reduction in root length over control.

iii. Nodules :

Nodule number slightly increased with 0.25% and 0.5% while higher concentrations of Mancozeb caused reduction in it at 6 h the exposure period.In general negative response was observed with all concentrations of Mancozeb with 12 h exposure period.

There are few reports available which describe the effect of pesticides on nodulation. Positive as well as negative effect of pesticides on nodulation was observed in different studies. Singh and Singh (1983) noticed that Carbendazim treatment stimulates nodulation in pea and soybean. Similar results were recorded by Kamble and Sabale (2002) in the case of carbendazim treated *Trigonella*. Vyas et al.(1985) have also reported that fungicides thirum, PCNB, carbendazim and metalaxyl stimulate the number of nodules in different cultivars of pea. Highest concentration of carbendazim reduced number of nodules in *Arachis hypogea* (L.) The lower concentration did not cause any deleterious effect on nodulation. (Bandopadhyay et al.1983). Chakraborty et al. (1985) also reported deleterious effect of dithane M- 45, Bavistin, captan and vitavax on nodulation in different plants. In the present study, Mancozeb treatment remarkably reduced the number of nodules especially at higher concentrations.

iv. Fresh Weight And Dry Matter :

At 6 h exposure period seed treatment and foliar spray of Mancozeb, all concentrations slightly increased fresh weight while remarkable reduction was observed with all concentrations of Mancozeb with 12 h exposure period seed treatment.

Pardeshi et al. (1989) reported stimulation in fresh weight in soybean seedlings with treatment of fungicides such as Mancozeb, bavistin, difoltan, vitavax and thirum.

Ganam and Indrani (1999) also noticed that bavistin, dithane and thirum seed treatment at the rate of 3 g /kg seeds increased fresh weight of *Glycine max* L. Merr. Kamble and Sabale (2002) also reported increase in fresh weight in *Trigonella* seedlings with Carbendazim treatment. Buta (1991) observed reduction in fresh weight in wheat with the treatment of paclobutrazol.

b. Effect of Anth (Chlorpyriphos and Cypermethrin) :

Effect of Anth (Chlorpyriphos and Cypermethrin) seed treatment and foliar spray on seedling growth of *Glycine max*(L.)Merr. is depicted in table 15.

i.Shoot Growth :

Seed treatment of Anth (Chlorpyriphos and Cypermethrin)with 6 h and 12 h exposure periods and foliar spray stimulated shoot length except negative effect of highest concentration.

ii. Root growth :

The seed treatment of Anth (Chlorpyriphos and Cypermethrin) at both the exposure periods and foliar spray positively influenced root length. Only highest concentration i.e. 2 % caused slight reduction.

Many workers have reported influence of insecticides on seedling growth of many plants. Carbofuran and phorate treatment stimulate shoot length and root length in soybean. (Saraswathi et al. 1996). These findings are in agreement to those of Sharma et al. (1997) who also have reported increase in root length of sugarcane treated with carbofuran and phorate. Agarwal and Bhanot (2000) have reported remarkable increase in root and shoot length of okra, after carbofuran and imidacloprid treatment. Kumar and Visalakshi (1983) have reported decline in epicotyl and hypocotyl growth in *Vigna mungo* L. treated with malathion.

iii. Nodule number :

All concentrations of Anth (Chlorpyriphos + Cypermethrin) at both exposure periods supressed the development of nodules in *Glycine max*(L.)Merr. Gradual decline in nodule number was observed with increase in concentration of Anth (Chlorpyriphos + Cypermethrin) at both exposure period seed treatment and foliar spray. Higher concentration of Anth with 6 h exposure treatment reduced the number of nodule drastically.

Table 15 : Effect of Anth (Chlorpyriphos and Cypermethrin) seed treatment and foliar spray on growth of *Glycine max* (L.) Merr.

Exposure Period	Pesticide	R L (cm)	S L (cm)	N D	L	F W (g)	D W (g)
6 h	Control	11.0 (±4.06)	15.9 (±2.41)	7 (±3.65)	5 (±1.41)	1.42	0.15
	Anth (Chlorpyriphos +Cypermethrin) (%v/v)						
	0.25	11.0 (±1.34)	17.4 (±1.39)	7 (±3.39)	7 (±1.34)	1.49	0.15
	0.5	14.0 (±1.41)	17.4 (±1.85)	2 (±2)	6 (±1.64)	1.53	0.16
	1	13.2 (±9.29)	18.5 (±2.35)	1 (±0.91)	5 (±0.1)	1.32	0.13
	2	8.6 (±2.07)	11.7 (±2.54)	1 (±0.7)	3 (±1.64)	1.13	0.10
12 h	Control	11.0 (±1.22)	15.2 (±1.22)	11 (±4.28)	7 (±1.34)	1.70	0.2
	Anth (Chlorpyriphos +Cypermethrin) (%v/v)						
	0.25	11.0 (±1.22)	16.4 (±1.29)	9 (±4.66)	6 (±1.64)	1.30	0.15
	0.5	12.0 (±2.0)	18.0 (±1.19)	5 (±2.92)	5 (±0.1)	1.36	0.16
	1	12.0 (±1.22)	16.0 (±1.58)	5 (±0.98)	5 (±0.1)	1.45	0.16
	2	10.2 (±3.56)	14.1 (±3.01)	3 (±1.19)	4 (±0.1)	1.36	0.18

SL : Shoot length
RL : Root length
ND : Nodule number per plant
FW : Fresh weight per plant
DW : Dry weight in per plant
L : Number of leaves per plant

(Values are mean of three replicates.)

PLATE 6

Influence of seed treatment and foliar spray of Anth (Chlorpyriphos + Cypermethrin) on growth of *Glycine max* (L.) Merr.
1. Control, 2. 0.25%, 3. 0.5%, 4. 1%, 5. 2%
A. 6 h seed treatment B. 12 h seed treatment

iv. Fresh Weight and Dry matter content :

In 6 h exposure period seed treatment and foliar spray with lower concentration of Anth (Chlorpyriphos + Cypermethrin) increased fresh weight, while decreased with higher concentrations. All the concentrations of Anth (Chlorpyriphos + Cypermethrin) at 12 h exposure period were highly phytotoxic inducing leaf burns. All concentrations of Anth at 12 h exposure treatments caused reduction in fresh weight of *Glycine max* L. Merr. seedlings after the seed treatment and foliar spray over control.

2. Photosynthetic pigments :

The effect of Mancozeb seed treatment and foliar spray on photosynthetic pigments of *Glycine max*(L.)Merr. is depicted in Table 16.

a. Chlorophylls :

Mancozeb treatment with all the concentrations at 6 h exposure period stimulated total chlorophylls. Maximum increase in total chlorophylls was observed at lowest concentration viz. 0.25 %. At the 12 h exposure period, the lowest concentration only increased total chlorophylls, while decrease in total chlorophyll was observed with increase in concentration of Mancozeb. Amount of chlorophyll 'b' was found positively influenced with compared to chlorophyll 'a'. At 6 h exposure period seed treatment, all the concentrations of Mancozeb and lower concentration at 12 h seed treatment stimulated content of chlorophyll 'a', while highest concentrations caused marginal decrease in chlorophyll 'a'. Seed treatment of Mancozeb at 6 h was found to be beneficial for chlorophyll synthesis with compared to 12 h exposure period.

The lowest concentrations of Mancozeb increased amount of chlorophyll 'b' while at higher concentrations viz. 1 % and 2 %, reduction was found in both the exposure period treatments. Ratio of chlorophyll 'a' to 'b' was found to be increased with respect to control when treated with different concentrations of Mancozeb at both the exposure periods. At 6 h exposure period seed treatment and foliar spray, all the concentrations of Mancozeb stimulated amount of carotenoids. Lowest concentration viz. 0.25 % stimulated content of carotenoid while other concentrations of treatment caused marginal increase in amount of carotenoid. Seed treatment with 12 h exposure period and

Table 16 : Effect of Mancozeb seed treatment and foliar spray on photosynthetic pigments of *Glycine max*(L.)Merr.

Exposure Period	6 h					12 h				
Pesticide	Chl a*	Chl b*	Chl a+b*	Chl a/b	carote noids	Chl a*	Chl b*	Chl a+b*	Chl a/b	Carot enoids
Control	55.3	67.3	122.6	0.82	14.4	57.82	66.3	124.1	0.87	15.2
Mancozeb (% w/v)										
0.25	77.45	85.4	162.8	0.91	20.0	62.63	66.8	129.3	0.94	15.2
0.5	70.25	82.3	144.5	0.85	17.6	60.63	63.1	123.7	0.96	15.2
1.0	62.63	66.8	129.3	0.94	17.2	58.36	61.7	120.1	0.95	14.8
2.0	60.63	63.1	123.7	0.96	15.2	56.22	59.7	115.4	0.95	14.2

Values are expressed in mg / 100 g fresh weight .

foliar spray of Mancozeb did not significantly influence carotenoid level. At 12 h exposure period seed treatment, higher concentration caused marginal reduction in carotenoid level.

The effect of Anth (Chlorpyriphos + Cypermethrin) seed treatment and foliar spray on the chlorophyll content of *Glycine max*(L.)Merr. is depicted in Table 17. At both the exposure period seed treatments and foliar spray of Anth (Chlorpyriphos + Cypermethrin), all the concentrations of Anth stimulated amount of chlorophyll 'a' and chlorophyll 'b'. Amount of chlorophyll 'b' was found positively influenced rather than that of chlorophyll 'a' especially at 6 h exposure period. Amount of total chlorophylls was also positively influenced by all the concentrations of Anth (Chlorpyriphos + Cypermethrin) with both the exposure period seed treatments especially at 6 h exposure

Table 17 : Effect of Anth (Chlorpyriphos and Cypermethrin) seed treatment and foliar spray on photosynthetic pigments of *Glycine max*(L.)Merr.

Exposure Period	6 h					12 h				
Pesticide	Chl a*	Chl b*	Chl a+b*	Chl a/b	Carotenoids	Chl a*	Chl b*	Chl a+b*	Chl a/b	Carotenoids
Control	55.3	67.3	122.5	0.82	14.4	57.82	66.3	124.1	0.87	15.2
Anth (Chlorpyriphos +Cypermethrin) (% v/v)										
0.25	66.9	71.74	138.6	0.93	17.6	66.77	72.89	139.6	0.92	13.6
0.5	76.18	85.85	161.9	0.89	20.0	64.5	71.54	136.0	0.90	17.2
1.0	84.92	104.5	157.1	0.82	23.2	59.09	71.33	133.4	0.87	17.2
2.0	68.91	75.39	144.2	0.91	18.0	59.09	66.3	124.9	0.90	26.4

Values are expressed in mg /100 g fresh weight.

period. At both the exposure period seed treatments and foliar spray of Anth (Chlorpyriphos + Cypermethrin), ratio of chlorophyll 'a' to 'b' was found to be increased over control.

Carotenoids :

All the concentrations of Anth (Chlorpyriphos + Cypermethrin) stimulated amount of carotenoids At both the exposure periods i.e. 6 h and 12 h and foliar spray, stimulation in carotenoid content was observed with all the concentrations of Anth (Chlorpyriphos + Cypermethrin). At 6 h seed treatment maximum value of carotenoid was observed at 1 % concentration while at 12 h maximum amount was observed at 2 % concentration.

Positive as well as negative effect of seed treatment and foliar spray with fungicides on photosynthetic pigments has been reported by several workers. Treatment of seeds with lower concentration of Mancozeb increased chlorophyll content while higher concentration reduced it in Bengal gram (Sugavanam, 1992). Kotasthane (1994) noticed that carbendazim, mancozeb and triforine sprays increase chlorophyll content in mustard leaves. Dhopte et al. (1995) observed slight increase in chlorophyll content in peanut sprayed with paclobutrazole. The foliage of triazole treated plants typically exhibits intense dark green colour (Fletcher et al. 2000). Lower concentrations of carbendazim were stimulatory in *Trigonella* (Kamble and Sabale 2002). Sharma and Thakore (2004) observed that systemic fungicides viz. iprobenphos, carbendazim, triophanate methyl and triadimefon when sprayed on chilli crop increased the chlorophyll content (Soni et al. 2006). These findings are in agreement to those of earlier workers (Singh and Kang 1983 ; Sankhla et al. 1990 ; Ghosh and Srivastava 1994 ; Dhopte et al. 1995 ; Chakravarty and Thakore 1997) who also reported increased production of chlorophylls in groundnut, peanut, *Quercus serrata*, tomato and cucurbits treated with oxithiins, benzimidazole, paclobutrazole, bavistin and metalaxyl.Thomas et al. (1995) observed reduction in chlorophylls and carotenoids in cucumber with the treatment of thirum, benomyl and triazole.

Stimulation in chlorophyll biosynthesis was observed with deltamethrin, cypermethrin and permethrin (Sukul and Handa 1989). In cucurbits, metalaxyl treatment elevated amount of chlorophylls (Chakravarty and Thakore 1997).Upadhyaya and Panda (2004) observed that treatment with monocrotophos showed reduction in chlorophyll and carotenoid content in tea seedlings. Foliar application of metacid -50 and dimecron stimulated chlorophyll content. (Kulkarni et al. 1989). Foliar application of metasystox and rogor reduced chlorophylls in ragi (Kumar and Khan 1982). Our results in *Glycine max* L. Merr. reveals the similar trend as reported by other workers.

3. Organic Constituents :

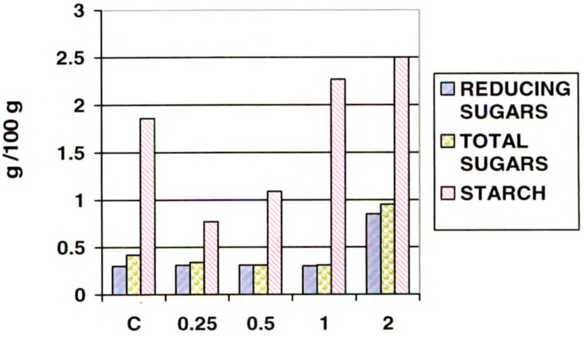
a. Carbohydrate content :

Effect of Mancozeb seed treatment and foliar spray on Carbohydrate content of *Glycine max*(L.)Merr. is depicted in Table 18. It is evident from the table that the amount of reducing sugars increased due to the seed treatment at both exposure periods and foliar sprays. Seed treatment with 6 h exposure period and foliar spray caused marginal increase in amount of reducing sugars with increase in concentration of Mancozeb, Increase in reducing sugars was notably observed over the control by highest concentration of Mancozeb (viz. 2 %). At 12 h exposure period also steady increase in reducing sugar content was observed after the foliar spray. About three fold increase in reducing sugars was observed at 2 % concentration. At 6 h exposure treatment, total sugar content was decreased with increase in concentration of Mancozeb. At higher concentration viz. 2 % amount of total sugars increased more than twice over the control. At 12 h exposure period, total sugar content was found increased after the foliar spray and exhibited an increase at all the concentrations of Mancozeb. Maximum amount of total sugars was observed at higher concentration viz. 2 % of Mancozeb at both the exposure periods.

Table 18 : Effect of Mancozeb seed treatment and foliar spray on carbohydrate content in *Glycine max*(L.)Merr.

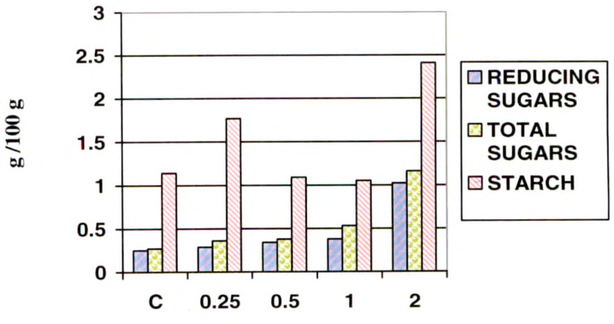
Exposure period	Pesticide	Reducing sugars*	Total Sugars*	Starch*	Total carbohydrates*
6 h	Control	0.30	0.42	1.86	2.28
	Mancozeb (% w/v) 0.25	0.31	0.34	0.77	1.11
	0.5	0.31	0.31	1.09	1.4
	1.0	0.30	0.31	2.27	2.58
	2.0	0.85	0.95	2.50	3.45
12 h	Control	0.25	0.27	1.14	1.41
	Mancozeb (% w/v) 0.25	0.29	0.36	1.77	2.13
	0.5	0.34	0.38	1.09	1.47
	1.0	0.38	0.53	1.05	1.58
	2.0	1.02	1.16	2.41	3.57

* All values are expressed in g / 100 g fresh weight .



Mancozeb (%)

Fig. 7 : Effect of 6 h seed treatment and foliar spray of Mancozeb on carbohydrate content in *Glycine max* (L.) Merr.



Mancozeb (%)

Fig. 8 : Effect of 12 h seed treatment and foliar spray of Mancozeb on carbohydrate content in *Glycine max* (L.) Merr.

At 6 h exposure period seed treatment and foliar spray, the lower concentrations of Mancozeb caused reduction in starch content and total carbohydrates, while at the higher concentrations it was increased.At the 12 h exposure period seed treatment and foliar spray, the highest concentration of mancozeb remarkably increased the starch content and total carbohydrates over control.

Influence of Anth (Chlorpyriphos + Cypermethrin) seed treatment and foliar spray on carbohydrate content of *Glycine max*(L.)Merr. is depicted in Table 19.

It is seen from the table that at both the exposure period seed treatment and foliar spray all the concentrations of Anth (Chlorpyriphos + Cypermethrin) caused decrease in reducing sugar content. Slight stimulation was observed at 1 % concentration of Anth (Chlorpyriphos + Cypermethrin) at 12 h exposure period seed treatment. In seed treatment of Anth (Chlorpyriphos + Cypermethrin) with 6 h and 12 h exposure period and foliar spray, the lower concentrations increased total sugars, while higher concentrations caused reduction in it.

In general, both the exposure periods and all concentrations of Anth (Chlorpyriphos +Cypermethrin) caused reduction in starch content and total sugars except slight increase with lowest concentration of Anth (Chlorpyriphos + Cypermethrin) at 6 h exposure period.

Ghosh and Srivastava (1994) have reported enhancement in the level of total sugars with carbendazim treatment in *Quercus serrata.* Foliar sprays of carbendazim positively influenced total sugars and starch content in *Allium cepa* (L.) varieties. Kamble and Sabale (2002) reported increase in total sugars and decrease in starch content with carbendazim application in *Trigonella*. Bhattacharya et al. (2001) observed decrease in carbohydrate content with foliar spray of carbendazim in rice.Sharma and Thakore (2004) reported increase in sugar content in chilli with application of systemic fungicides viz. iprobenphos, carbendazim and triadimefon. Singh and Kang (1983) have noticed increased production of total sugars in groundnut plants treated with oxithiins and benzimidazole fungicides. These findings are similar to those of Marshall et al. 1991 and Siddiqui 1997 who have also reported increase in sugar contents of soybean, peanut and brinjal crops treated with systemic fungicides.

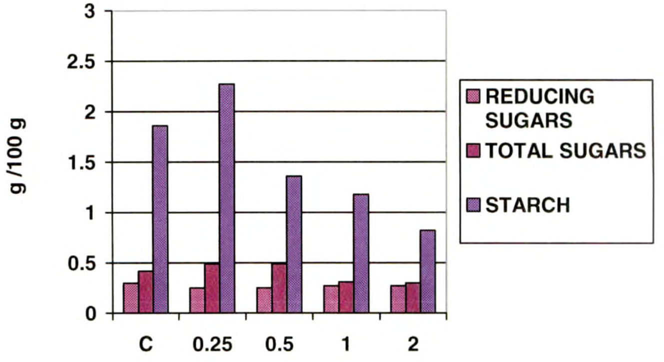
Paul et al. (1995) noticed decline in fructose and glucose content in tobacco leaves with increased concentration of monocil.Kamble and Sabale (2002) reported that application of monocrotophos decreased the sugar content while favoured starch accumulation. Sundararaj et al. (1993) have reported that insecticides viz. cypermethrin, monocrotophos and combination of Cypermethrin+Chlorpyriphos (1:10) did not significantly increased reducing sugars, non reducing sugars and total sugars.

Table 19 : Effect of Anth (Chlorpyriphos + Cypermethrin) seed treatment and foliar spray on carbohydrate content in *Glycine max*(L.)Merr.

Exposure period	Pesticide	Reducing sugars	Total sugars	Starch	Total carbohydrates
6 h	Control	0.3	0.42	1.86	2.28
	Anth(Chlorpyriphos +Cypermethrin) (% v/v)				
	0.25	0.25	0.49	2.27	2.76
	0.5	0.25	0.49	1.36	1.85
	1.0	0.27	0.31	1.18	1.49
	2.0	0.27	0.30	0.82	1.12
12 h	Control	0.25	0.27	1.13	1.40
	Anth(Chlorpyriphos +Cypermethrin) (% v/v)				
	0.25	0.18	0.24	1.09	1.33
	0.5	0.24	0.27	1.00	1.27
	1.0	0.26	0.20	0.91	1.21
	2.0	0.17	0.20	1.73	1.93

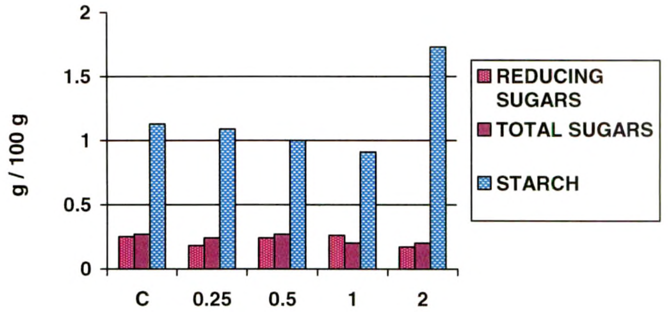
All values are expressed in g /100 g fresh weight.

Fig. 9 : Effect of 6 h seed treatment and foliar spray of Anth (Chlorpyriphos+ cypermethrin) on *Glycine max*(L.)Merr



Anth (Chlorpyriphos+ cypermethrin) (%)

Fig. 10 : Effect of 12 h seed treatment and foliar spray of Anth (Chlorpyriphos+ cypermethrin) on *Glycine max*(L.)Merr



Anth (Chlorpyriphos+ cypermethrin) (%)

b. Total polyphenol content :

Effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment and foliar spray on polyphenols in *Glycine max*(L.)Merr. is depicted in table 20.

Table 20 : Effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment and foliar spray on Total soluble proteins and Total polyphenols in *Glycine max*(L.)Merr.

Exposure Period	6h		12 h	
Pesticide	Total soluble proteins	Total polyphenols	Total soluble proteins	Total polyphenols
Control	4.3	0.38	6.2	0.45
Mancozeb (% w/v)				
0.25	6.7	0.51	9.6	0.61
0.5	8.0	0.61	9.1	0.66
1.0	8.4	0.66	9.1	0.76
2.0	11.6	0.81	9.4	0.51
Anth (Chlorpyriphos +Cypermethrin) (%v/v)				
0.25	7.3	0.45	7.8	0.45
0.5	8.4	0.56	8.4	0.63
1.0	8.7	0.63	9.1	0.71
2.0	11.3	0.76	12.0	0.61

Values are expressed in g / 100 g fresh weight .

It is evident from the table that Mancozeb seed treatment with both the exposure periods of Mancozeb with foliar spray caused increase in polyphenol content with increase in concentration.

Similar results were obtained with the treatment of Anth (Chlorpyriphos + Cypermethrin) showing increase in the polyphenol content with increase in concentration of pesticide at both the exposure periods.

Polyphenols are the aromatic compounds formed during secondary metabolism. Phenolic compounds function as donors or acceptors of hydrogen ions in oxidation - reduction reactions. The phenolic compounds in some instances affect fundamental processes such as photosynthesis, plant water relations, protein synthesis and membrane permeability (Rice, 1979 ; Dark et al. 1975 ; Glass and Dunlop, 1974). Polyphenols play an important role in disease resistance.

Many workers have studied the effect of different pesticides on polyphenol content in different plants. Reilly and Klarmann (1972) reported stimulation in soybean treated with maneb, benomyl and nabam. An accumulation of total polyphenols was observed in blackgram during methyl parathion treatment (Thirumaran and Xavier, 1987). Sharma and Thakore (2004) reported increase in phenol content in chilli plants with the application of systemic fungicides as compared to untreated plants. These findings are in agreement to those of earlier workers (Gautam et al.1984 ; Marshall et al. 1991 ; Mohmed et al. 1988 ; Singh and Kang 1983 and Siddiqui 1997) who also reported increase in phenol content of soybean, peanut, onion, groundnut and brinjal crops treated with systemic fungicides. Bavistin treatment increased polyphenols while polyphenols did not exhibit much change with monocrotophos treatment in *Trigonella* (Kamble and Sabale 1995).

Sundararaj et al. (1993) reported decrease in phenolic content of cotton plants treated with spray application of fenvalerate and cypermethrin. Methyl parathion and phosphomidon sprays stimulated polyphenol synthesis in tomato, gaur and okra (Kulkarni et al. 1989). According to Wang (1961) insecticides stimulate polyphenol content which may minimise pathogen attack. Thus it can be said that pesticides make the crop plants resistant to pest attack by accumulation of phenols.

In the present study, both fungicidal and insecticidal treatment stimulated polyphenol synthesis in *Glycine max* L. Merr. which might make the plant resistant to pests.

c. Total soluble proteins :

The effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment and foliar spray on total soluble proteins in *Glycine max*(L) Merr. is depicted in table 20.

It can be seen from the table that protein content of soybean plant was increased with the application of both the pesticides viz. Mancozeb and Anth (Chlorpyriphos + Cypermethrin) at both the exposure periods viz. 6 h and 12 h. Concentration wise increase in protein content was observed with the seed treatment of both the pesticides i.e. Mancozeb and Anth (Chlorpyriphos + Cypermethrin) with both the exposure periods.

Both positive and negative effects of pesticides on protein content have been reported in different plants. Mancozeb treatment at 250 - 500 ppm increased protein content in bengal gram while 1000 ppm decreased it (Sugavanam 1992). Ghosh and Srivastava (1994) noticed increased protein content in *Quercus serrata* after foliar spray treatments of carbendazim. Ahmed and Siddiqui, 1995 ; Siddiqui et al. 1997 ; Siddiqui and Khan, 2001 also have reported increased protein content in crops like *Capsicum annum, Vigna radiata, Solanum melanogena* and *Pennisetum americanum* treated with systemic fungicides. Kamble and Sabale (2000) have reported increase in protein content in *Trigonella* seeds treated with bavistin and monocrotophos. Kalebere and Sabale (2000) have reported that foliar sprays of carbendazim positively influenced protein content in onion. Pratibha and Gupta (2006) studied interactive effect of fungicides, insecticides and SO2 on maize plants. They have reported increase in total soluble proteins in response to all the pesticides. Soni et al. (2006) have recorded that triazoles, paclobutrazol and bayleton showed low protein content at all the growth stages with respect to control grown *Sesamum indicum* plants.

Sukul and Handa (1989) while studing effect of synthetic pyrethroids ; deltamethrin, permethrin, cypermethrin and fenvalerate in chickpea noticed that these insecticides did not significantly influence protein content of chickpea. Sundararaj et al. (1993) also could not observe any adverse effect on protein content in cotton plants after the application of cypermethrin, ethofenfox, monocrotophos and combination of Cypermethrin + Chlorpyriphos (1 : 10). Insecticide decamethrin spray increased the level of soluble proteins in leaves of cotton (Dominick and Mahanasundharam, 1992). Singh (1994) reported declined protein accumulation in in vivo grown *Vigna unguiculata* plants treated with Bayleton. In present investigation, both the pesticides viz. Mancozeb and Anth (Chlorpyriphos + Cypermethrin) stimulated total soluble proteins in *Glycine max* L. Merr. These findings are similar to those of Sugavanam 1992 ; Ghosh and Srivastava

1994 and Siddiqui and Khan, 2001.

5. Enzyme Studies :

a. Protease [EC 3.4.3.2] :

The effect of Mancozeb seed treatment and foliar spray on the activity of protease in *Glycine max*(L.)Merr. is reported in table 21.

It is evident from the table that Seed treatment of Mancozeb and Anth with 6 h and 12 h exposure periods and foliar spray caused general increase in activity of enzyme protease over control.

Asirselin et al. (1998) observed stimulation in protease activity at lower concentration of bavistin in *Arachis hypogea*. Kamble and Sabale (2002) observed slight inhibitory effect of carbendazim on the activity of protease in *Trigonella*. Fungicides such as chloramphenicol and cycloheximide have been reported to inhibit protease activity in maize and Cicer (Srivastava et al. 1972 ; Bose et al.1982). Kamble and Sabale (2002) observed slight inhibitory effect of carbendazim on the activity of protease in *Trigonella.*

In many studies, negative effect of insecticides was observed on protease activity in many plants. Kamble and Sabale (2002) recorded decline in protease level after foliar sprays of monocrotophos in *Trigonella*. Malathion and Aldrin treatments were found to reduce protease activity in linseed and mustard (Singh et al. 1997 ; Sengupta et al. 1986). In the present investigation, amount of protease in the seedlings was found increased with increase in dose of Anth (Chlorpyriphos + Cypermethrin) in both the exposure periods.

b. Nitrate Reductase [EC 1.6.6.1] :

The activity of Nitrate Reductase in response to Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment and foliar spray in *Glycine max*(L.)Merr. is depicted in table 21. It is evident from the table that the seed treatment with 6 h exposure period and foliar spray of Mancozeb caused increase in nitrate reductase activity at lowest concentration while all other higher concentrations caused inhibition with compared to control. The 12 h exposure period seed treatment and foliar spray of Mancozeb stimulated the enzyme activity with all concentrations.In both the exposure periods viz. 6 h and 12 h seed treatments and foliar spray, negative influence of Anth

(Chlorpyriphos + Cypermethrin) on Nitrate reductase activity was observed. Concentration wise reduction in Nitrate reductase activity was observed with the seed treatment of Anth (Chlorpyriphos + Cypermethrin) with both the exposure periods and foliar spray.

Table 21 : Effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment and foliar spray on the activity of Protease and Nitrate Reductase in *Glycine max* (L.) Merr.

Exposure period Pesticide	6 h		12 h	
	Protease*	Nitrate Reductase @	Protease*	Nitrate Reductase @
Control	18.38	93.6	22.65	51.6
Mancozeb (% w/v)				
0.25	21.94	99	28.35	90.6
0.5	28.35	92.4	31.2	90.6
1.0	28.35	92.4	36.18	84.6
2.0	35.47	64.8	38.32	65.4
Anth (Chlorpyriphos +Cypermethrin) (% v/v)				
0.25	28.35	61.8	26.93	51.6
0.5	37.61	42.0	46.86	49.8
1.0	26.21	33.0	56.83	42.0
2.0	21.23	19.2	61.10	33.0

* Values are expressed in µg tyrosine liberated / h /g
@ Values are expressed in in µg NO_2 produced h^{-1} g^{-1} fresh weight .

Pulver and Ries (1973) observed increase of ten fold in Nitrate reductase activity in maize treated with simazine. Ghosh and Srivastava (1994) observed that lower concentration of bavistin enhanced Nitrate reductase activity while higher concentrations were inhibitory in *Quercus serrata.* Kamble and Sabale (2002) noticed continuous rise in Nitrate reductase activity with increased concentration of carbendazim and monocrotophos. Nitrate reductase activity increased in response to fungicides dithane and ridomyl and insecticide endosulfan treatments in maize plants (Pratibha and Gupta 2006). Sairam et al.(1989) reported that triadimefon enhanced Nitrate reductase activity in wheat plants. In canola leaves, triazoles promoted the activity of Nitrate reductase enzyme (Srivastava and Fletcher 1992). Soni et al. (2006) observed that triazoles enhanced Nitrate reductase activity in in vivo grown *Sesamum indicum* plants at all the three growth stages.

c. Acid phosphatase [3.1.3.2] :

Acid phosphatase activity as observed with Mancozeb seed treatment and foliar spray in *Glycine max*(L.)Merr. is recorded in table 22 .

It can be seen from the table that seed treatment with 6 h exposure period and foliar spray of Mancozeb caused concentration wise inhibition of acid phosphatase activity. Similar trend was recorded with Anth (Chlorpyriphos +Cypermethrin) seed treatment and foliar spray. Both the pesticides caused increase in acid phosphatase activity with 12 h exposure period seed treatment and foliar spray.

Kamble and Sabale (2002) reported stimulation of acid phosphatase activity in *Trigonella* with the treatment of carbendazim. Sengupta et al. (1986) reported decline in acid phosphatase activity in wheat by malathion. Griarifreda et al. (1993) reported inhibition of acid phosphatase activity in potato by carbaryl. A high level of acid phosphatase observed in present study with both the pesticides at 12 h seed treatment and foliar spray which indicated an increased hydrolysis of phosphorus esters and also a higher rate of metabolism resulting in stimulation of growth of plants.

d. Catalase [EC 1.11.1.6] :

The effect of Mancozeb and Anth(Chlorpyriphos + Cypermethrin) seed treatment and foliar spray on catalase activity in *Glycine max(L.)Merr.* is depicted in table 22. It is evident from the table that the seed treatment of Mancozeb with 6 h and 12 h exposure period and foliar spray caused in general increase in enzyme catalase activity. While Anth(Chlorpyriphos + Cypermethrin) seed treatment with 6 h exposure period and

Table 22 : Effect of Mancozeb and Anth (Chlorpyriphos + Cypermethrin) seed treatment and foliar spray on the activity of Acid phosphatase and Catalase in *Glycine max*(L.) Merr.

Exposure period	6 h		12 h	
Pesticide	Acid Phosphatase*	Catalase @	Acid Phosphatase*	Catalase @
Control	44.4	5.0	34.0	2.86
Mancozeb (% w /v)				
0.25	43.2	9.21	44.0	3.20
0.5	40.0	11.02	42.2	5.81
1.0	39.6	11.93	40.0	8.26
2.0	24.0	14.28	24.0	6.53
Anth (Chlorpyriphos +Cypermethrin) (% v /v)				
0.25	38.0	5.9	44.8	6.60
0.5	39.6	3.2	41.6	5.21
1.0	40.0	3.0	41.2	3.0
2.0	40.0	0.8	40.0	2.5

* Values are expressed in Δ OD / h / g fresh weight.
@ Values are expressed in mg H_2O_2 broken down /min/ g fresh tissue

foliar spray caused in general concentration wise inhibition of activity except lower concentration. The 12 h exposure period of seed treatment and foliar spray caused concentration wise increase in activity except the highest concentration i.e. 2 %.

Catalase is an oxidative enzyme which catalyses breakdown of H_2O_2. Catalase has got more affinity for H_2O_2 and is involved in regulation of H_2O_2 and is involved in regulation of H_2O_2 level in plant tissue. Kamble and Sabale (2002) reported that carbendazim caused increase in catalase activity with increased dose of carbendazim while higher concentration of monocrotophos caused marginal decrease in catalase activity in *Trigonella.*