CHAPTER - 4

EFFECT OF PESTICIDES ON SEED GERMINATION, SEEDLING GROWTH AND ENZYMES
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The present chapter deals with the studies of seed germination and seedling growth of brinjal and tomato in relation to ethion and cypermethrin pesticides.

4.2. RESULTS:-

4.2.1 SEED GERMINATION:-

The effect of cypermethrin and ethion on seed germination of brinjal and tomato. Ethion in all concentration inhibite seed germination in both the crops. The germination after 4 days was 52% in tomato and 58% in eggplant in highest concentration of ethion. At minimum concentration i.e 0.01 % the germination after 4 days was 96% in tomato and 98% in brinjal. Other concentration viz. 0.15% and 0.25% also inhibite the seed germination in both the crops. Ethion was found to be more toxic to tomato. Cypermethrin at lowest concentration i.e 0.01% significantly stimulate the seed germination in both the crops. The seed germination after 4 days was 95% and 96% in control set of tomato and brinjal but in lowest concentration of cypermethrin the seed germination after 3 days was 99% and 96% in brinjal and tomato. Cypermethrin in 0.15 %, 0.25%, and 1.00% concentration inhibite the
germination in both the crops but maximum inhibition was noticed at the highest concentration. At this concentration the seed germination after 4 days was 72% and 65% in brinjal and tomato. Ethion was found to be more toxic than cypermethrin.

4.2.2 Radicle Growth:

Radicle length was measured after 1, 3, 5 and 7 days of germination. Ethion at every used concentration significantly inhibits the length of radicle of tomato and brinjal (Table, Fig 4.1 and 4.3). Cypermethrin on the other hand stimulates the radicle growth of tomato and brinjal in lowest concentration (Table, Fig 4.2 and 4.4).

Table, Fig 4.1 show that ethion at used concentration inhibits the growth of radical of tomato seedling. Maximum inhibition was observed at highest concentration (1.00%) . On this concentration the length was 43% less than the control after 5 days of germination, but maximum inhibition was noticed after 3 days of germination where the growth was 66.17% than the control. At the minimum concentration viz. 0.01% the maximum inhibition was observed after 5 days of germination i.e 10.11% than the control. Ethion at every concentration inhibits the growth of radical of brinjal where highest concentration show maximum inhibition after 1 days of germination i.e 64.19% than the control (Table, Fig 4.3). The inhibitory effect of ethion was concentration dependent and maximum inhibition was observed after 5 days of germination, ethion inhibits the growth of radical of tomato more than the radicle of brinjal.

Table, Fig 4.2 and 4.4 show that cypermethrin at 0.01% stimulates the growth of radicle of tomato and brinjal. In tomato maximum stimulation was after 3 days of germination where the length of radicle was 10.66% than control (Table, Fig 4.2). In brinjal maximum stimulation was observed after 7 days of germination where the length of r
radicle was found 9.07% than the control (Table, Fig 4.4). The range of stimulation was 5 to 7.9% than the control in tomato. The range of stimulation was 4 to 6.6% than the control of in brinjal. Cypermethrin was found more stimulatory in the tomato at 0.01% concentration. At higher concentration maximum inhibition was observed in tomato. Where the radical was 59.34% than the control after 1 days of germination. The maximum inhibition after 3 days was 58.77 %. The data was found statistically non-significant. The inhibition in both the crops was continued up to 7 days after germination but at every used concentration of both the pesticides the inhibition was maximum after 1, 3 days of germination in tomato and brinjal. Cypermethrin was found to be more stimulatory at lower concentration in the brinjal radicle than the tomato radicle.

Table, Fig 4.1 and 4.3 show that the ethion was more toxic than the cypermethrin. Tomato was more responsive than brinjal for both the pesticides. The two pesticides inhibits the length of radicle at higher concentration of ethion and cypermethrin pesticides.

4.2.1 Plumule Growth:-

Plumule length was measured after 4 and 6 days of germination. Ethion at every concentration inhibites the plumule length of tomato and brinjal (Table, Fig 4.5). Ethion at highest (1.00%) concentration was found more toxic to tomato. After 4 days and 6 days of germination the plumule length was 62.5% and 53.96%. However inhibition at lowest concentration was 9.35% and 45.41% than the control after 4 days and 6 days after germination (Table, Fig 4.5). In comparison to tomato ethion was toxic to brinjal where maximum inhibition at highest concentration was 72.85% and 64.58% than the control after 4 days and 6 days of germination . In lowest concentration maximum inhibition was 3.87% than the control after 4 days of germination. Ethion inhibite the plumule length of tomato more than brinjal and maximum inhibition was reported after 4 days of germination.
Cypermethrin in 0.01% concentration significantly stimulate the plumule length of tomato and brinjal (Table, Fig 4.6). The stimulation was observed to be more in brinjal than the tomato. Cypermethrin in higher concentration inhibits the plumule length of tomato and brinjal. Maximum inhibition for both crops was noticed at highest concentration. Where the inhibition was up to 52.79% than the control in case of tomato and 58% than the control in case of brinjal. Maximum stimulation was up to 9.41% and 6.63% than the control in case of tomato and brinjal.

Like radicle there was a concentration dependent inhibition of plumule length. If we compare both than we find that both the pesticides inhibit the radicle of both the crops more than plumule. But cypermethrin was more promotory to the plumule than the radicle of tomato and brinjal.

4.2.1 Number Of Rootlet:-

Table, Fig 4.7 and 4.8 show different trends of rootlet development. Ethion and cypermethrin both at 0.25% and 1.00% concentration inhibit the number of rootlet in both the crops. The maximum inhibition in both the crops was observed after 4 days of germination. Ethion at 1.00% concentration show maximum inhibition 75.67% and 79.62% than the control in tomato and brinjal. Cypermethrin show maximum inhibition at 1.00% concentration i.e. 67.2% and 66.66% less than the control in tomato and brinjal. Ethion at 0.01% concentration significantly stimulate the rootlet development where rootlet up to 10.18% more than the control in tomato and 8.79% more than the control in brinjal. Ethion and cypermethrin was found be more stimulatory at 0.01% concentration. Cypermethrin on the other hand in both tomato and brinjal significantly inhibit the rootlet development at lowest concentration where maximum inhibition was 12.5% and 14.82% than the control in tomato and brinjal. Cypermethrin in 0.25% and 1.00% concentration inhibit the number of rootlet in both the crops
Cypermethrin at 0.01% and 0.15% concentration increases the number of rootlet than the control in tomato and brinjal.

4.2.5. **Seedling Dry Weight:**

Seedling dry weight was increased after 3, 5 and 7 days of germination. Ethion at every concentration inhibite the dry weight in both tomato and brinjal. Maximum inhibition was observed after 5 days of germination. Ethion at highest 1.00% concentration show maximum inhibition in dry weight of both tomato and brinjal seedling. At this concentration the maximum inhibition was 76% and 62% than the control in tomato and brinjal. At lowest concentration the maximum inhibition was 12% and 10% than the control in tomato and brinjal.

Cypermethrin on the other hand show different trend. At lowest concentration cypermethrin accelerates seedling dry weight of both tomato and brinjal. Maximum induction was observed after 5 days of germination although induction like inhibition continued up to 7 days after germination. Maximum acceleration was observed 10.5 % and 8.5% than the control in tomato and brinjal, cypermethrin in other concentration significantly inhibite the seedling dry weight of tomato and brinjal. Maximum inhibition was found at the highest concentration where there was reduction in tomato and brinjal to the tune of 59% and 48% than the control. Ethion show maximum inhibition at every used concentration both pesticides were found more toxic to tomato seedling, cypermethrin in lowest concentration promoted seedling dry weight of tomato more than the brinjal.

4.2.5 **Enzymes:**

Amylase activity in experimental seedling (both treated and control) was found maximum at 5 days old seedling (Table, Fig 4.9 and 4.10).
Ethion at every concentration inhibite the activity of amylase in both tomato and brinjal (Table, Fig 4.9). The maximum inhibition in every used concentration was noticed after 5 days and than less inhibition at 7 day. Ethion at highest concentration (1.00 %) show maximum inhibition (46.66 % and 59.18%) less than control in tomato and brinjal. The maximum stimulation at lowest concentration (0.01 %) of ethion was 4.76% and 6.84% than the control in tomato and brinjal. Cypermethrin at 0.01% concentration stimulate amylase activity in both tomato and brinjal (Table, Fig 4.10) but maximum stimulation was obtained in 5 days of old brinjal seedling. In the rest of concentration cypermethrin significantly inhibite amylase activity of tomato and brinjal seedling and found more toxic to tomato seedling enzymes.

Protease activity in both treated and control seedling was found highest in 5 days old seedling (Table, Fig 4.11 and 4.12). Ethion like amylase inhibites protease activity but inhibition for both crops tomato and brinjal was comparatively low. At highest concentration maximum inhibition was 48.48% and 49% than the control in tomato and brinjal. Maximum activity was noticed in lower concentration seedling. Cypermethrin in lowest concentration stimulate the activity of protease there after inhibition takes place. The maximum inhibition was found at highest concentration (1.00%) i.e. 34.88% and 41.52% than the control in tomato and brinjal seedling.

5’-Nucleotidase activity was also found highest in 5 days old seedling of treated and control sets (Table, Fig 4.13 and 4.14). Maximum inhibition was found in 5 days old seedling of tomato and brinjal at highest concentration of both pesticides (ethion and cypermethrin). In tomato inhibition was 33% and 57.68% than the control by ethion and cypermethrin. Cypermethrin at lowest concentration stimulate the activity of 5’-nucleotidase i.e 14.71% and 15.75 % than the control in tomato and brinjal. Cypermethrin at higher concentration inhibite the activity of 5’-nucleotidase 27.96% than the control in brinjal.

Ribonuclease also followed the same trends with maximum activity in
5 days old seedling of both treated control plants (Table, Fig 4.15 and 4.16). Unlike other enzymes in cypermethrin pesticides at lowest concentration stimulate the activity of RNase but stimulation was more than the ethion pesticides, other used concentration inhibite the activity of ribonuclease in tomato and brinjal. Maximum inhibition was obtained in 5 days of seedling. Ethion at highest concentration inhibite the activity of enzyme up to 48% and 60.25% than the control in tomato and brinjal and ethion at lowest concentration stimulation the activity of enzyme up to 3.69% and 6.58% than the control in tomato and brinjal. Cypermethrin found to be stimulatory in lowest concentration but inhibit the activity in other concentration (Table, Fig 4.16). The maximum inhibition of activity in both tomato and brinjal by ethion pesticides. Cypermethrin at lowest concentration stimulate the activity of enzyme up to 5.9% and 10.34% than the control and highest concentration inhibit the activity of enzyme up to 45.59% and 57.69% than the control in tomato and brinjal.

All the enzymes followes the same trend of activity. Ethion in both tomato and brinjal at all concentration inhibites the activity of every enzymes significantly but ethion was found to be more toxic to the tomato seedling than the brinjal seedling. Cypermethrin at the lowest concentration stimulates the activity of all enzymes in both tomato and brinjal but it was more stimulatory to tomato seedling than the brinjal. Cypermethrin in other (0.25% and 1.00%) concentration was found to be highly toxic to both the seedling but cypermethrin at 0.15% concentration moderately toxic in both the crops. Like ethion it was more toxic to tomato seedling than the brinjal.

4.2 DISCUSSION:-

Seed germination in tomato and brinjal inhibite by all the concentration of ethion and cypermethrin. This is in agreement with the investigation of Dhawan et al., (2004) was observed highest concentration of ethion
and cypermethrin decrease the cotton yield in *Gossypium hirsutum*. They observed 56% germination by 1% concentration in mung bean Trivedi et al., (1983) and Gupta et al., (1983) was found inhibitory action of endosulfan in varying concentration from 0.01% to 1.00%. Singal and Bhanot (2001) was found the cypermethrin at 0.04% and 0.06% concentration on seed germination of green gram and found increased the percentage of abnormal seedling and higher doses of cypermethrin increased the percentage of hard and dead seeds of green gram. We observed cypermethrin at lower concentration stimulate the seedling and seed germination and higher concentration inhibite the seed germination and seedling growth this agreement with (Kumar and Nath, 2003). Rao (1985) also reported inhibition of germination in gram by endosulfan and Chandra and Mathur (1985) was found inhibition of germination in cowpea and urd seeds by malathion. Chandra and Mathur (1985) was observed concentration dependent inhibition of imbibition of water, inhibition of translocation of reducing sugar from cotyledons to axis and decreased absorbing/holding capacity of axis and these changes led to the inhibition of seed germination. Banerjee and Bera (1969) was observed effect of systemic insecticides on paddy seeds during germination. Bangar and Puri (1981) was observed seed germination and seedling growth of cotton.

Kumar and Nath (2004) was found monocrotophos, cypermethrin at 0.006%, carbaryl, deltamethrin, endosulfan and malathion concentration of pesticides on pigeon pea and the minimum loss in seed weight (2.89%) and the highest grain yield (24.85% and 18.28 %) were observed in plots treated with endosulfan, cypermethrin, fenvalerate, deltamethrin, carbaryl and malathion. The lowest grain yield was recorded in the control plots. Beevers and Splittstoesser (1968) reported protein and nucleic acid metabolism in germinating pea. Mote (1978) was found 100% germination in 0.03% concentration of pesticides treated seeds of pea but at 1% germination was inhibite to the toxic level. Similar report have been made by Kumar et al., (1987). Benjamini (1986) was observed effect of carbofuran on seed germination, initial development of seven crops. Bhardwaj et al., (1988) was recorded inhibition of elongation of pollen tube by pesticides. Chandra and Mathur (1985) was observed
responses of *Vigna mungo* Hepper to treatment of malathion and carbendazim and effect on germination physiology and seedling growth. Singh, Tiwari and Maurya (2004) reported cypermethrin at 0.025 % and some other pesticides concentration used on seed germination and lentil of vigour and they found on various all the pesticides treatments were better seed germination than the control at lower concentration. Various other workers have also noticed stimulatory action of pesticides in lower concentration but inhibitory in higher concentration (Agarwal et al., 1986; Mahla, 2001; Chaudhary and Dashad, 2002). Somshekhar and Sreenath (1987) was found dose dependent response of pea and black gram seeds treated with benelate. Bandyopadhyay and Mukherjee (1983) was found effect of some seed treating with fungicides on nodulation of legumes.

Prasad (1986) reported in mungbean recorded inhibition of amylase reducing sugar contents and inhibition of germination by calyx in, on the other hand increased in amylase reducing sugar contents followed by increase in germination.

Radicle length was inhibite by every used concentration of both the pesticides (ethion and cypermethrin) except lowest concentration of cypermethrin which stimulate the growth. Mote (1978) also found the similar result in dimethoate treated seedling.Gupta et al., (1983) was found inhibite growth of radical of mung bean seedling even in 0.1% concentration of endosulfan. Several other workers have also reported stimulating response of various pesticides in low concentration followed by concentration dependent inhibitory effects (Saxena t al., 1972 ; Goel, 1982 ; Gupta, 1986 and Pathk and Mukherjee, 1986). Agarwal et al., (1986) was found effect of cythion pesticide on seed germination, seedling growth of *Triticum aestivum*. Sengupta et al., (1986) and Campbell and Marrison (1987) also found the inhibitory effect are in pasture species. Pawar et al., (1979) reported effect of systemic insecticides on the germination and seedling development of okra (*Abelmoschus esculentus* ). Gawaad et al., ( 1972 ) was found effect of some soil insecticides on germination, dry weight and nodulation in *Trifolium alexandrium*. Gentle et al., (1978) reported cucumber pollen germination and tube elongation inhibited or
reduced by cypermethrin. Kumar, Sharma and Jain (1987) was observed bionegative effects of organophosphorus pesticides on germination and seedling growth of *Vigna sinesis*. Systemic insecticides on germination and growth of urd seeds (Saxena et al., 1972).

Plumule like radicle show the similar response to ethion and cypermethrin. Compbell and Morrison (1987) reported 22-78% inhibition by tetrapon in pasture species. Somshekhar and Sreenath (1987) was found that inhibition of plumule growth. Ramesh and Sabarinathan (2006) was found root growth and nodulation characteristics of cowpea in situ soil and moisture conservation nutrient management practices under rainfed alfisols ecosystem and result revealed that on root growth (length, dry wt.), nodulation characteristics (number and dry wt.) and grain yield of coepea were increased significantly. Nitrogen and zinc interaction on nodulation of french bean (Srivastava and Pant , 2006). Agarwal et al.; Pathak and Mukherjee (1986) reported dual effect i.e. promotion in low concentration and inhibition in high concentration of monocrotophos and sevin.

As far as comparative effect of pesticide on radicle and plumule is concerned, there was more inhibition of root than the shoot at every concentration of both the pesticides i.e. ethion and cypermethrin in both the crops i.e. tomato and brinjal. The more inhibition occurring in roots might be due to the direct contact with toxic compounds. Inhibitory action of pesticides may be due to inhibition of nucleic acid contents as observed by Badra and Ibrahim (1987). During seed germination seedling depend on storage tissues for reserve food to be used through the action of hydrolyzing enzyme may also be reason of growth inhibition.

Promotion of growth seedling in low concentration of pesticides may be due to increase in level of RNA, DNA and amino acid, protein study by Zama and Hatzios (1986). Prasad (1986) and Pathak and Mukherjee (1986) reported increase in activity of amylase leading to increased amount of reducing sugars. Pathak (1986) found increased growth is mediated through increased activity of hydrolyzing enzymes.
Vats and Nandal (1994) was used root dip treatment of tomato seedling against *Meloidogyne javanica*. Ahuja and Kaur (1985) was found effect of rogor insecticides on super oxide dismutase, peroxidase, lipid peroxidase non protein SH group in germination mung bean.

Seedling dry weight was found to be maximum 7 days old seedling of treated and control as well. The maximum weight in these seedlings was due to maximum growth of 7 days old seedlings. Ethion decreased seedling dry weight of tomato and brinjal at every used concentration. Chandra and Mathur (1985) was found the inhibition was due to comparatively less growth of the treated seedlings.

Prasad (1986) and Chandra and Mathur (1985) reported amylase hydrolyses the reserve food material of seed and changes reducing sugar level. Young and Varner (1959) was recorded increase in amylase activity during germination of pea. Rao (1985) was observed inhibition of amylase by dimethoate and endosulfan in high concentration. Pathak and Mukherjee (1986) reported inhibition of amylase by many pesticides and dimethoate lowest concentration promoted the activity then there was continuous inhibition (Gupta, 1986 and Prasad, 1986). Rajender (1986) was observed gibberellic acid release amylase from lysosome. Dubey and Rani (1990) was observed influence of NaCl salinity on the behaviour of protease amino peptidase and carboxy peptidase in rice and tomato seedlings. Sammon (1998) reported protease inhibitors and carcinoma of the esophagus. Golstryan (1965) was observed the method of determining the activity of hydrolytic enzyme in soil. Hassanei and Ahmed (1999) was found isoenzyme expression during root and shoot formation in *Solanum nigrum*. The use of enzyme ELISA test for quantitative detection of potato virus Y in potato and other test plants (Ismail, 1998). Inn and Smith (1967) was observed the localization of enzymes in cotyledons of *Pisum arvense* during germination. Chandra and Mathur (1986) was observed increase in amino acid and protein contents of treated seedlings. Royer et al., (1974) thus the possibility of pesticidal control of protease activity through protease inhibitors can not be ruled out. Agarwal and Tayal (1984) was observed in vitro inhibition of alpha amylase and protease
by nematicides in *Cicer arietinum*. Chrispeels and Varner (1967) reported gibberellic acid enhanced synthesis and release of alpha amylase and RNase by isolated barley aleurone layers. Daussant and Renard (1972) was observed immunilogical comparison of amylase in germinating and developing wheat seeds. Tanifuji et al., (1978) was found development of RNase activity in embryonic axes of germinating pea seeds. Baumgartner and Chrispeels (1976) was observed partial characterization of protease inhibitor which inhibits the major endopeptidase in the cotyledons of mung bean. Naito et al., (1979) was observed the effect of benzyladenine on changes in nuclease and protease activities in intact beans leaves during aging. Jacobson and Varner (1967) was found increase activity by the novo synthesis of protease from amino acid released by proteolysis from storage protein. This is may be correlate with the finding of Gomma (1977).

5’- Nucleotidase catalyzes catabolic cleavage of pyrimidine 5-monophosphatase formation of purine nucleotides (Wasternack, 1982). Nucleotidase represent a large group of enzymes which are also potential of nucleotide metabolism indicated by the existence of a mechanism for regulation of their activity. Sengupta et al., (1986) was found enhanced activity of nuclease activity by malathion accompanied with low DNA contents.

Ribonuclease is an endosoluble enzymes attacks on RNA and forms intermediated products, 2,3-cyclic phosphates and end products 3- P mono and oligonucleotides reported by Farkas (1982) . Chromoplast development in ripening tomato fruit identification of CDNAs for chromoplast targeted proteins and characterization of a CDNA encoding a plastid localized low molecular weight heat shock protein observed by Moore and Lawrence (1997). Roberts and Lord (1979) was observed the maximum activity was found in 3 days old seedlings. Pathak and Mukherjee (1986) reported the RNase activity may increase simultaneously with an increase in RNA contents. Mukherjee (1986) were also reported stimulation of activity in low concentration and inhibition in high concentration of sevin. Pandey (1989) reported promotion in activity is possible mediated through the action of pesticides
on protein synthesis due to rupturing of the lysosomal membrane. Barker et al., (1974) was observed the development of RNAse and acid phosphatase during germination of *Pisum arvense*. Murray (1980) was observed functional significance of acid phosphatase distribution during embryo development in *Pisum sativum*. Pandey (1989) reported changes induced by carbaryl in the carbohydrate metabolism and phosphatase enzyme of sugarcane top shoot borer.*L. novella*. The pesticides may cause disorganization of plasamalemma of organelles (Tripathi et al., 1982).

The inhibition in RNAse activity may be due to impairment of RNA and protein for details refer chapter 5.