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CHANGES IN HERBACEOUS COMMUNITY PATTERN, GROWTH AND BIOMASS ACCUMULATION UNDER SINGLE AND COMBINED TREATMENT OF CHLORPYRIFOS AND MALATHION INSECTICIDES

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Abstract

Organophosphate pesticides are highly toxic insecticides, causing sever neurotoxic impacts on pests. Use of pesticides in agriculture is increasing to protect the food commodities from pests to fulfill the increasing food demand. Indiscriminate use of insecticides may cause negative impacts on community structure and biodiversity. The present study was conducted to assess the community composition, canopy cover, growth and biomass accumulation in herbaceous community under individual and combined treatments of Chlorpyrifos (CP) and Malathion (MT) with respect to untreated control. Jaccard and Sorenson similarity indices showed more than 50% similarity in communities under different treatments. *Coronopus didymus* (L.) Sm. and *Oxalis corniculate* L. were most dominant species in community under all the treatments. The numbers of plant species were 18, 14, 13 and 11, respectively in control, CP, MT and CP+MT treatments. Canonical discriminant analysis discriminates the effects of treatments on the growth parameters where combined treatment showed antagonistic effect, while individual treatments have similar effects. This study indicates that Chlorpyrifos and Malathion insecticides at recommended dose have potential to change species composition, growth and biomass of herbaceous community.

Key words: Chlorpyrifos, Malathion, herbaceous community, growth, biomass accumulation

Introduction

In India use of pesticides is about 3 percent of the world uses and rate is increasing each year (Ravi and Fulekar, 2018). Many pesticides are banned, but many more numbers of pesticides are registered for agricultural uses. Uttar Pradesh state shows highest pesticide's consumption, where uses of Malathion and Chlorpyrifos are more than seven million tons during 2005-2010 (India for safe food, 2020). Organophosphate insecticides are widely used due to its low toxicity and persistent nature as compared to organochlorine (Costa, 2006). Organophosphate insecticides are lipophilic in nature and hence adversely affect the living cell membrane (Videira *et al.,* 2001).

Ever increasing population rise and consequent demand for food, directly or indirectly leads to an increase in the use of pesticides for more crop production. But repeated uses of pesticides lead to change in biodiversity pattern, its deterioration as well as accumulation in higher trophic levels causing bio-magnification (Sitaramaraju *et al.,* 2014). Use of pesticides causes beneficial effects on one side by reducing crop losses, preventing from vector diseases and improving the quality of food by killing the pests, but on the other side causes hazardous effects by reducing the soil fertility, increasing soil contamination, impacting on non-target organisms and contaminating the food web (Aktar *et al.,* 2009).

Use of pesticides may cause many acute and chronic residual effects on ecosystem such as negative impacts on non-target organisms even on some keystone species, which are important part of ecosystem structure and function, and promote non-essential species which may dominate due to alteration in prey and predator relationship (Sitaramaraju *et al.,* 2014). Increasing frequency of insecticide uses severely affected the diversity of wild plants, carabid species and bird diversity compared to the organic farming (Geiger *et al.,* 2010).An important grass, *Lolium perenne* and a forb, *Centaurea jacea* showed reductions in their growth and biomass under Chlorpyrifos insecticide treatment compared to the control due to insecticide-mediated soil feedback in form

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ofdecrease in plant nutrition and nutrient cycling (Eisenhauer *et al.,* 2010). There are variations in effects of pesticides according to their doses, plant species and the environmental variables. The low concentration of orthophosphate insecticides were found good for the growth, but at higher concentration adverse effects on plants were apparent (Singh *et al.,* 2018). There is a strong relation between concentration and effects of pesticides on plants as doses below recommended or sometimes the recommended can cause adverse effects (Shakir *et al.,* 2016).

Thepresent study was conducted to understand the effects of selected insecticides individually and in combination on community structure, growth characteristics and biomass accumulation in naturally growing plant species under semi-natural condition. The insecticides used were organophosphate insecticides, Malathion and Chlorpyrifos, which are commonly used in agriculture as well as for domestic purposes.

Materials and methods

Study site

This experiment was conducted in the Botanical garden of the Department of Botany, Banaras Hindu University, Varanasi (a district of Uttar-Pradesh state), situated at co-ordinates 25º 18' N, 82º 03' E and 80.71 m above sea level of eastern-Gangatic plain of Indian subcontinent during October, 2018 to January, 2019. These months are post-monsoon season, when moisture content is not a constrain for plant growth. Maximum temperature during the experimental period varied from 24.51ºC to 34.17 ºC and minimum temperature from 8.56ºC to 14.16 ºC. Mean relative humidity ranged from 85.41% to 86.73%. The total mean rainfall was only 4.7 mm during January.

Experimental design

This experiment was conducted in plastic pots of 30×16 cm (diameter and height) size, having 10 kg of soil by weight. The treatments were Chlorpyrifos (CP), Malathion (MT), combination of CP+MT and control (without insecticides). The dose was 0.6mM for Chlorpyrifos in 10 kg soil and 5.9 mM for Malathion. The doses were recommended and used by farmers. The doses of Chlorpyrifos and Malathion in mixture were same as given during individual treatment. Tap water was used to irrigate the pots and same amount of water was given in each pot. Surface soil from 5-15cm was collected from the Botanical Garden and characterized before filling in the pots at the start of the experiment. The soil colour was brownish yellow with pH of 7.3, conductivity of 4.6 mS cm^{-1}, total nitrogen of 0.18 % and total organic carbon of 0.91%. Soil for different treatments including control were prepared atone place and then after stabilization devided into four heaps of different treatments. The pots were randomly distributed at a gap of 1.0 meter. The pots were left in ambient natural condition to colonize.

Plant sampling and analysis

Plant sampling was conducted after three months of the exposure of insecticides. Number of herbaceous plant species in each pot was counted. The similarity coefficient was calculated using Jaccard and Sorensen indices (Chao *et al.,* 2005). Jaccard similarity index is a relation between common and uncommon species within two communities and total number of plant species within those communities, while Sorensen index shows resemblance between two communities with ratio of double of common species and cumulative of all plant species in both the communities.

Plants were harvested in January when most of the plants were in the reproductive stage. Each plant was collected in separate poly bag and morphological parameters such as number of leaves, number of branches, shoot length and root length were measured. Canopy cover was taken by using a software (Canopy Cover Free version 1.03) with the help of a phone camera after keeping pots on brown paper to a fixed distance. For total biomass, harvested plants after washing to remove adhered soil particles, were kept in an oven till a constant weight was achieved. Then the weight of plants was taken by using digital balance.

Stastical analysis

All the data were first analyzed for normality and homoscedasticity. ANOVA test was performed to check the significance of effects of each treatment on different parameters considering individual pot as a statistical unit. Different parameters under each treatment were then tested by discriminant analysis to identify whether the selected parameters were able to separate the significant variability due to insecticide exposure. Parameters taken for the analysis are total shoot length, total root length, total number of leaves, total number of branches and total biomass as whole plant's statistical mean with respect to the treatments. Discriptive statistics and univariate analysis were done with the data of measured parameters in the study. These statistical tests were performed by using SPSS (SPSS Inc. version 21.0, IBM Crop, Armonk, NY, USA) software and presentations were done using Sigma Plot 11.0 (Systat software Inc) software.

Results

The number of plant species naturally colonized the pots is given in table 1. All the species were not present in all the treatments. The numbers of naturally colonized plant species were 18, 14, 13 and 11, respectively in control, CP, MT and CP+MT treatments. *Eclipta alba*, *Eleusine indica*, *Psorelia corilifolia* and *Trianthema portulacastrum* were only present in control table 1. *Echinocloa colonum* and *Phyllanthus asperulatus* were only not present in CP+MT treatment. *Cyperus rotundus* was only not present in MT treatment table 1. *C. didymus* was present in all treatments with the highest number. *O.corniculate* showed highest number under combined CP+MT and MT treatments and *Nicotiana plumbaginifolia* showed highest number in CP treatment. *C. didymus* was most dominant plant species in differently treated pots followed by *O. corniculate* and then *N. plumbaginifolia* table 1. Least numbers of plant species were *Digitaria ciliaris*, *Euphorbia hirta*, *E. colonum*, *Cyanodon dactylon*, *C. rotundus*, *Medicago polymorpha* and *Melilotus indica* table 1. Both the similarity indices showed similar pattern between different treatments with respect to presence/absence of plant species Fig. 1. Values of both similarity indices varied from 0.5 to 0.9, representing more than 50% similarity among different treatments with respect to naturally colonizing plant species Fig. 1.

Table 1: Number of plant species under control and different insecticide treatments.

Name of plant species	Con-trol	CP	MT	CP+MT
Ageratum conyzoides L.	2	1	2	3
Coronopus didymus(L.) Sm.	18	17	16	23
Cyanodon dactylon (L.) Pers.	2	1	1	1
Cyperus rotundus L.	2	3	-	1
Digitaria ciliaris(Retz.) Koeler	1	3	2	3
Echinocloa colonum(L.) Link	1	1	3	-
Eclipta alba (L.) Haskk.	4	-	-	-
Eleusine indica(L.) Geartn	2	-	1	-
Euphorbia hirta L.	1	1	1	1
Launea procumbens (Roxb.) Ramayya Rajgopal	4	3	3	5
Mecardonia procumbens (Mill.) Small	5	3	3	3
Medicago polymorpha L.	2	1	-	-
Melilotus indica(L.) All.	2	1	1	5
Nicotiana plumbaginifolia Viv.	4	7	3	6
Oxalis corniculate L.	8	10	16	16
Phyllanthus asperulatus Hutch.	3	5	5	-
*Psorelia corilifolia*L.	1	-	-	-
*Trianthema portulacastrum*L.	1	-	-	-

CP: Chlorpyrifos; MT: Malathion; CP+MT: Chlorpyrifos + Malathion.

Percentage cover area under different treatments was highest in CP+MT (20.5%) and lowest in MT (11.6%) Fig. 2. There is no significant variation in total root length of plant species under different treatments Fig. 2. Total shoot length of control treatments was 31.7% higher compared to MT, 13.6% higher than CP and 16.0 % higher than CP+MT treatments Fig. 2. For total number of branches, CP showed significant increase of 23% compared to control and 20.8% than combined treatment. MT treatment had no significant difference compared to the control. A significant difference was recorded for total number of leaves between MT and CP+MT treatments Fig. 2. CP+MT showed highest number of leaves and MT the lowest. Similarly, for total biomass, CP+MT showed highest and MT showed the lowest value Fig. 2. Growth parameters such as total biomass, shoot length, number of leaves and number of branches showed significant effects of insecticide treatments as shown by F and p-values ($p<0.05$) in table 2.

Discriminant analysis showed that total number of leaves and total numbers of branches were identified as major discriminating parameters, which were able to explain most of the variance under different insecticide treatments table 3. Based on discriminant analysis, 70.4% of variance in plant parameters was explained by discriminant function 1 and 2. On the basis of plant

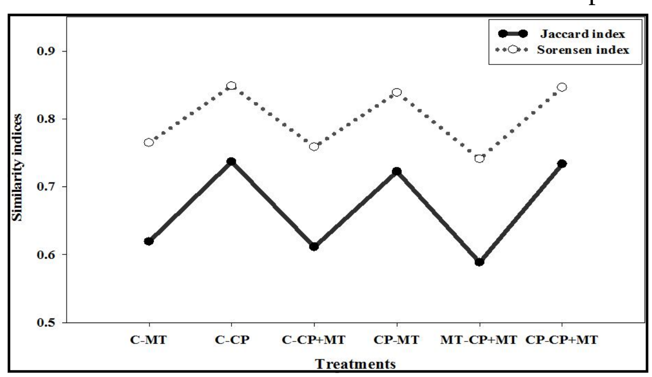

Fig. 1. Trends of Jaccard and Sorensen similarity indexvalues between different treatments.

Table 2: Results of Univariate analysis on different plant parameters under different treatments.

Parameters	F-values	p-value
Total biomass	2.974	0.034*
Total root length	1.120	0.342^{NS}
Total shoot length	3.485	0.017*
Total number of leaves	4.061	0.008**
Total number of branches	2.878	0.038*

Level of significance :* = $p<0.05$ and ** = $p<0.01$ and NS = non-significant.

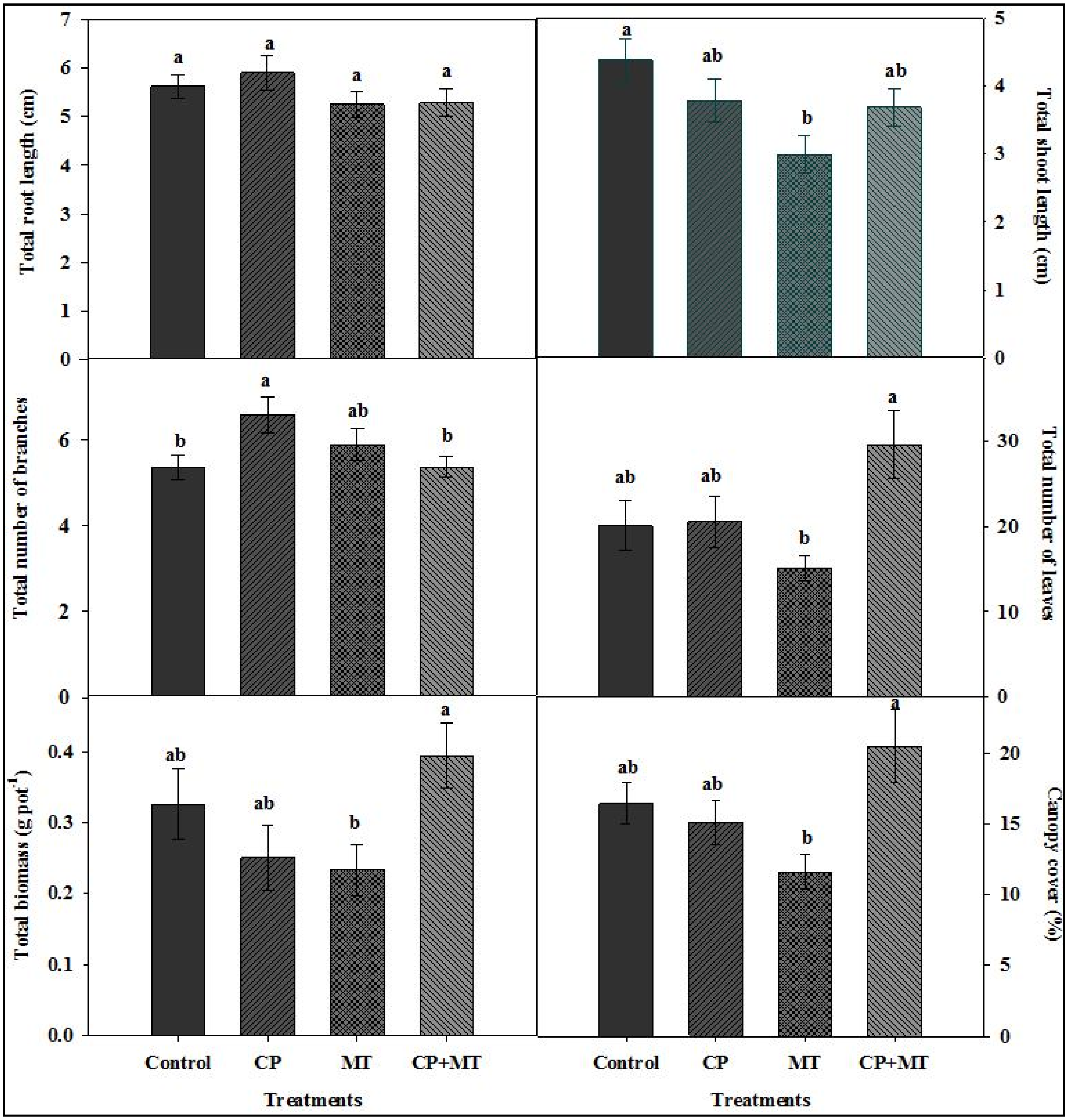

Fig. 2: Total shoot length and root length (cm), numbers of leaves and branches, biomass (g per pot) and canopy cover (%) under different treatments. Values are mean of replicate pots with standard error. Different alphabetical letters on the bars indicate significant differences at $p<0.05$.

parameters taken for the study, canonical discriminant function represented effects of different insecticide treatments. CP and MT were grouped together and overlapped, whereas CP+MT was well separated with control, CP and MT. Control plants showed partial overlap with all the three, but was closer to CP+MT. All the observed parameters were able to separate each group distinctly in scattered plot of discriminant analysis Fig. 3.

Discussion

The present study showed that there are variations in number of plant species under different treatments. Maximum number of species was found in control followed by CP and MT and least in CP+MT treatments. Further, there were 10 plant species common in all the treatments and control in which *C. didymus* followed by

Table 3: Discriminant analysis on parameters to test the equality of group means with univariate analysis.

Parameters	Wilks' Lambda	F	p-value
Total root length	0.971	0.966	0.412[NS]
Total shoot length	0.968	1.191	0.317[NS]
Total number of leaves	0.855	6.031	0.001**
Total number of branches	0.906	3.699	0.042*
Total Biomass	0.955	1.677	0.176[NS]

Level of Significance:*= p<0.05, **= p< 0.01 and NS= non-significant

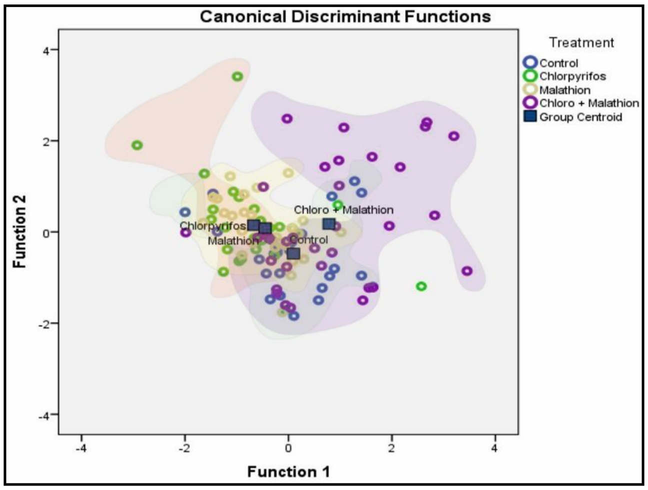

Fig. 3. Scatter plot for representing distribution of parameters under different treatments based on canonical discriminant function analysis.

O. corniculate and *N. plumbaginifolia* were most dominating herbaceous plant species. The dominance of *C. didymus* may be ascribed to its invasive nature with enormous seed production property. *O. corniculate* has robust rooting system, which helps in its successful establishment. The plant species which are less in number or not present under insecticide treatments compared to the control showed their susceptibility to the insecticides. In a weed management study under rice and wheat cropping system, herbaceous plant species such as *C. didymus*, *Melilotus alba*, *Medicago denticulate*, *Phalaris minor*, *Rumex dentatus* and *O. corniculate* were found to dominate in the community after herbicide application in the field (Chhokar *et al.*, 2007). More dominance of *Oxalis* sp. in maize crop growing field compared to other annual and perennial wild plants was attributed to its more vegetative reproduction by underground bulbs, tubers and stolon after herbicide application in the field (Thomas, 1991). *C. rotundus* growth was affected by combined treatment of pesticides at different doses despite it's dominance as wild herbaceous plant species under natural condition with underground tuber with high growth rate and lower light compensation point (Gilreath and Santosh, 2005). In the present study, *C. rotundus* was present more in number under CP treatment compared to CP+MT treatment.

The similarity between communities of different treatments reflects their environmental and soil nutrient conditions. Verma *et al.*, (2015) represented that changes in pH and nutrients in soil cause modification in species diversity due to species susceptibility and tolerance of specific type of plant species. The dominant species showed good growth under application of insecticides due to reduced herbivory, which helps in vigorous growth and high biomass accumulation. Under combined treatment, number of plant species was lowest, while dominant species showed highest numbers. This trend clearly suggests that community simplification occurred under CP+MT treatment and dominant species took over the advantage and increased their number. Wardle and Barker (1997) found that under application of insecticides, the biotic pressure is reduced in a natural plant community of grasses and wild herbaceous plant species, leading to high biomass production. Nutrient availability is important component, which is significantly affected by the use of insecticides in the soil (Das and Mukherjee, 2000).

Change in branching pattern in herbaceous plants under stressed condition occurs due to alteration in shoot height to alter the number of leaves as an adaptation for photosynthesis to continue other metabolic processes. In the present study, individual and combined treatments showed different magnitude of effects where individual treatments showed overlapping while combined treatment showed distinct effect. In CP+MT treatment, the effect of one insecticide may have been neutralized by the effect of other Fig. 3. Sometimes organophosphate pesticides show their enhancing effects by restraining the process of detoxification of other chemicals (Munkegaard *et al.*, 2008). Combined treatment of Di-syston and Phorate showed additive effect on oats, while combination of Diuron, Di-syston with phorate showed synergistic effects (Nash, 1967). In this study, application of MT caused negative effects on total biomass and height of herbaceous community, but Brown *et al.*, (1987) found no significant effect of MT on plant growth parameters. Growth inhibition and reduction in biomass accumulation under pesticides application in field directly or indirectly relates to changes in metabolic processes in plants (Igbedioh, 1991). Total biomass and canopy cover were highest under CP+MT, suggesting that reduction in competition caused vigorous growth and biomass accumulation in dominant species, leading to higher production compared to the control, where more species participated in community formation.

CP+MT treatment showed higher number of leaves, biomass and canopy cover, whereas MT showed the lowest compared to the control. CP treatment caused higher number of branches and shoot length, but lower numbers of leaves and biomass compared to the control. These trends may be ascribed to differences in individual and combined effects of the insecticides where combined treatment showed antagonistic effect. Organophophate pesticides, Disulfoton and Terbufos when given in combination with Metribuzin cased phytotoxic effects on soybean, while Metribuzin combined with Aldicarb and Carbofuran did not cause any significant toxicity (Hammond, 1983). No significant effects on growth and yield and no visible symptoms of injury were found on soybean under combined treatments of Chlorimuron, Clomazone, Imazaquin and Imazethapyr (Krausz *et al.*, 1992).

Conclusions

Individual and combined treatments of Chlorpyrifos and Malathion caused profound impacts on species composition, growth, canopy cover and biomass of herbaceous community after three months of insecticides exposure. Dominance of *C. didymus*, *O. corniculate* and *N. plumbaginifolia* more under treatments showed their tolerance to insecticides. The plant species (*T. portulacasprum*, *P. corilifolia*, *E. alba and E. indica*) not present under insecticide treatments, but present under control condition showed their sensitivity to insecticides. *E. colonum* and *P. asperulatus* are found sensitive towards combined treatment. The study further showed that application of the insecticides reduce herbivory, so growth, canopy cover and biomass accumulation of dominant species increased in the community. It may be concluded that insecticide tolerant species enhanced their performance in view of lower competition, which led to highest biomass accumulation under combined treatment.

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