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**RESEARCH ARTICLE**

# Effect of mulching on diversity and abundance of natural enemies associated with brinjal (*Solanum melongena* L.) crop in Mawathagama, Kurunegala (IL1)

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***ABSTRACT***

*Misuse of insecticides has negative impacts on the environment and human health; hence, a search for non-chemical alternatives for insect pest control is a timely need. Natural enemies play a significant role in suppressing insect pest populations in crops. Mulching has an impact on the diversity of natural enemies in soil, but it has not been adequately documented locally. Thus, this study was conducted to examine the influence of mulches on natural enemies associated with the pests of the brinjal crop. Four mulch materials viz. rice straw, gliricidia (Gliricidia sepium) leaves, weed residues, and black colour polythene, as the treatments, were laid on plots of brinjal crop. Unmulched plots, with and without weeds, were also used as controls. Experimental design was RCBD with three replicates. Soil inhabitants were caught using pitfall traps and soil sampling was undertaken up to 10 cm depth. Foliage insects were collected using sweep netting. The total number of natural enemies significantly ($F_{(5,120)} = 5.928$, $P<0.05$) varied between the treatments. The highest mean number of natural enemies was recorded in rice-straw mulch (2.50 plot$^{-1}$), followed by weed residues (1.19 plot$^{-1}$), gliricidia (1.10 plot$^{-1}$), and black polythene (0.10 plot$^{-1}$). Unmulched plots left with weeds had a higher mean number of natural enemies (1.29 plot$^{-1}$) than the plots without weeds (0.43 plot$^{-1}$). Pest abundance was significantly ($F_{(5,120)} = 3.897$, $P < 0.05$) different between treatments. The highest abundance was recorded in unmulched plots (25.30 plot$^{-1}$), followed by weed residues (7.95 plot$^{-1}$), gliricidia (7.76 plot$^{-1}$), straw (6.50 plot$^{-1}$), and black polythene (2.0 plot$^{-1}$). The study concluded that rice-straw mulching favours natural enemies, while black polythene reduces insect pest abundance. Further, the presence of weeds on plots enhances the abundance of natural enemies.*

***Keywords**: Brinjal, insect pests, mulch materials, natural enemies of insect pests*

## INTRODUCTION

In Sri Lanka, vegetables are cultivated over 84,191 ha, mainly in Nuwara Eliya, Badulla, Kandy, and Puttalam districts. Up country vegetables are produced mainly in Nuwara Eliya and Badulla districts while low-country vegetables are cultivated in Puttalam, Anuradhapura, and Hambantota districts (AgStat, 2018). Among the constraints of vegetable production, the management of insect pests is a significant component. Vegetable farmers primarily rely on insecticide applications to control insect pests through integrated pest management which is highly promoted by the relevant authorities. Misuse of insecticides is common among vegetable farmers (Sumudumali *et al.*, 2021). Insecticide spraying is associated with many consequences, such as resistance development in pest populations, resurgence of pest populations, occurrence of secondary pest outbreaks, human health hazards, and impacts on other beneficial organisms (Sharma and Singhvi, 2017). Therefore, it is necessary to minimize insecticide use in vegetable production; hence, there is a need to explore the possibility of using safer and eco-friendly non-chemical methods for insect pest control in vegetable crop cultivations.

Many of the Sri Lankan farmers are aware of alternative pest control methods such as crop rotation, intercropping, manual removal of pests *etc.* Still, only a very low percentage of farmers are aware of the biological and cultural control strategies in pest management (Sivarajah, 2019). Mulching is one of the cultural strategies that affects population levels of pests and natural enemies (Shirgure *et al.*, 2003). Mulching materials could be categorized as living and non-living types, the latter being further divided into synthetic and organic mulches (Alyokhin *et al.,* 2020). Organic mulches include organically derived materials such as straw, hay, sawdust, gliricidia, weed residues *etc*. Cover crops are also used as a living mulch grown before or with the main crop and maintained throughout the growing season as a living ground cover. As inorganic mulches, different polythene films can be used, which are derived from petroleum-based products (Gill and Goyal, 2014).

According to the type of mulch, insect population and diversity can vary (Sunderland and Samu, 2000). Some plant diseases can be controlled by applying mulches to the soil. Transparent polythene sheets are used for soil disinfestations/sterilization and solar heating of soil to control soil-borne pathogens (Chen *et al.*, 2021). In addition, transparent polythene mulches have a repellent effect on aphids and whiteflies, as the polythene mulch reflects Ultra Violet (UV) light (Arogundade *et al.*, 2019). It also reduces the virus-transmitting vector population and reduces virus disease incidences (Shah *et al.*, 2020). Flying aphids and thrips respond to visual stimuli and contrast the soil background with plant foliage to locate the host plant. This can interrupt by applying mulches to the soil (Alyokhin *et al.*, 2020).

Not only the insect pest species but also the natural enemy population is also affected by mulching. Mulching improves soil conditions which is favorable for the living of soil insects (Kader *et al.*, 2017). They provide shelter for natural enemies. Therefore, biological control of insect pests is possible with mulching (Mochiah *et al.*, 2012). The natural enemy population associated with soil varies according to the mulch type (Alyokhin *et al.*, 2020). Some organic mulches, such as hay, act as a nutritious food source for insects. Thus, the natural enemy population is relatively high in organic mulches (Buck *et al.,* 2000). Living mulches increase the soil-dwelling insect diversity of agricultural fields, thus impacting the effectiveness of natural enemies (Prasifka *et al.,* 2006).

In Sri Lanka, farmers use different mulches without considering their effect on natural enemies and pests (Kumara and De Silva, 2019). Identifying the most effective mulch that controls pests is essential to increasing the natural enemy population as an eco-friendly approach to suppress the pest population in vegetable ecosystems. But most of the research work related to mulching and the abundance of natural enemies and pests in brinjal fields have been done in different countries except Sri Lanka. Therefore, this study was conducted with the objective of evaluating the effect of different types of mulches on the diversity and abundance of natural enemies over time in the brinjal ecosystem.

## MATERIALS AND METHODS

The field experiment was conducted in a vegetable field at Mawathagama area, Kurunegala district, Sri Lanka, from March to May 2021. The field was situated in the $IL_1$ Agro-ecological region.

### Field selection plot arrangement and planting of the crop

The experimental site was not previously sprayed with any agrochemical, and the land was an abandoned field surrounded by unmanaged natural vegetation. Weeding was done manually without using any herbicide. Eighteen plots, each 3 x 1 m in size, were prepared and the space between two adjoining plots was 0.5 m. Brinjal (*Solanum melongena* L.) seedlings (20 d old) were transplanted at one seedling/hill at 0.9 × 0.6 m spacing as per the recommendation of the Department of Agriculture. There were two rows of plants, having eight plants in each plot, accordingly.

### Treatment (mulch) application

Four mulches, *i.e.*, rice straw, weed residues, gliricidia (*Gliricidia sepium*) leaves, and black polythene mulch, were applied as treatments. Furthermore, unmulched-unweeded and unmulched-weeded plots were also included as the control treatments. Altogether, there were six treatments in the experiment. Rice straw, weed residues, and gliricidia (*Gliricidia sepium*) leaves were selected for the experiment since those materials are commonly available and usually used by the farmers. Also, polythene mulch was used to compare the results obtained by naturally available mulches. Mulching was done using each mulch material only once soon after planting brinjal on plots. Also, fresh, natural mulches were applied, unless it may create biased results as the decomposition rate of each mulch material varies due to different C: N ratios (Goh and Tutua, 2004). Each mulch was laid on inter rows of brinjal, thoroughly covering the entire plot, which was randomly selected from each block. Treatments were thus assigned in randomized complete block designs (RCBD) with three replicates. The amount of each mulch material required to cover one square meter area varied with the material type and was pre-determined as shown in Table 1.

**Table 1:** Amount of mulch materials applied as treatments.

| Mulch type | Amount of mulch (kg) | |
|---|---|---|
| | For 1 $m^2$ area | For entire plot (3 $m^2$ area) |
| Rice straw | 2.5 | 7.5 |
| Gliricidia leaves | 1.76 | 5.3 |
| Weed residues | 4.26 | 12.8 |
| Black polythene | 0.5 | 1.5 |

**Crop establishment**

Brinjal plants were irrigated daily for the first 4-5 d, then every 3-4 d, depending on rainfall. No insecticides were applied during the experimental period. Compost was applied to plants once in two weeks, as recommended. Weeds were removed by hand-pulling in both weeded and mulched plots.

**Field assessments**

***Sampling of insects***

Pitfall traps were used for capturing insects that are dwelling or crawling on the soil. Each trap consisted of two cups (200 mL), which were fitted on one over the other. The cup size was 7 cm (top diameter) x 5 cm (bottom diameter) x 10 cm (height). Both cups were buried in the soil, leveling the cup's rim with the soil surface. The surrounding soil was made smooth, simulating the undisturbed soil. Each trap was half-filled with a concentrated salt solution to avoid decaying trap content. The roof was installed over the trap to prevent rainwater accumulation. Pitfall traps were set out in the middle of each plot at the beginning of the experiment, and traps were run for 48 h in the field and emptied. For example, traps were set every Monday morning at the same time and ran for 48 h. After 48 h, samples were collected and closed the lid until the next week Monday. Therefore, samples were collected once every week. The day of emptying the trap was recorded as the sampling date. Between the collection periods, plastic lids were put on traps. Trap content was strained using a cotton cloth, and insects were collected into containers.

Soil sampling was carried out once in every week by obtaining two soil samples from each plot using a soil sampling cylinder which is 10 cm in height and 7 cm in diameter. Samples were collected into separate polythene bags, and insects found there were separated from the soil and put them into containers. Soil samples were then put back into respective plots where samples were taken. Sweep netting was done to collect pests and natural enemies found on foliage. Some pests, like aphids (Family Aphididae), were counted while they were on leaves. Natural enemies like coccinellid beetles (*Coccinella* spp.) were immediately identified, and photographs were taken and released to the experimental field from where they were collected. Only a few coccinellid beetles (*Coccinella* spp.) of different species were preserved for identification.

### *Insect identification*

Collected insects were transferred and stored in glass bottles having 70% ethanol and labeled with collection details. Insects were identified by examining them under the dissecting microscope (magnification: 10 x 2) while consulting identification keys, sketches, and photographs available online.

### *Data analysis*

A separate analysis was carried out for each insect family. Chi-square analysis was performed for Formicidae, Aphididae, Cicadellidae, Membracidae families, and order Araneae, where at least 20 or more insects were present. The abundance of insects associated with treatments was analyzed using a one-way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (DNMRT) at $P \leq 0.05$ significance level. All statistical analysis was performed by SAS software (9.0 version). Shannon-Weaver diversity index (H') was calculated to compare the diversity of insects in tested treatments.

## RESULTS AND DISCUSSION

### Assessment of diversity and abundance of natural enemies and pests

Of the collected insect specimens, natural enemies and insect pests were identified (Figure 1 and Figure 2).

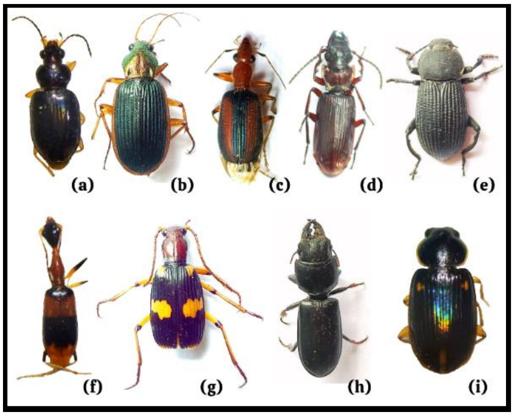


**Figure 1**: Different Genus of Carabidae Family and Tenebrionidae Family: (a) *Bembidion* spp. (b) *Chlaenius* spp. (c) *Drypta* spp. (d) *Goniotropis* spp. (e) *Menearchus* spp. (f) *Ophionea* spp. (g) *Pheropsophus* spp. (h) *Scarites* spp. (i) *Stenolophus* spp.

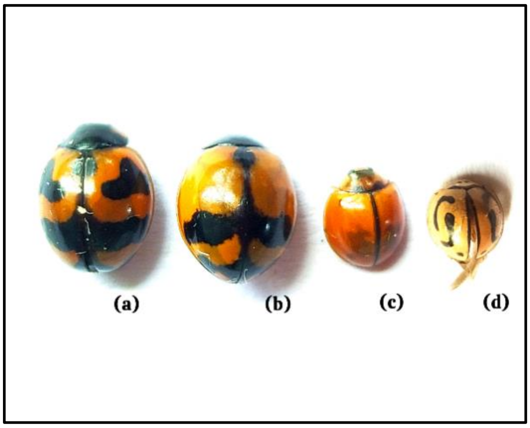


**Figure 2:** Different Genus of Coccinellidae Family: (a) *Coccinella transversalis,* (b) *Coccinella octomaculata,* (c) *Micraspis discolor,* (d) *Anegleis cardoni*.

As shown in Figure 3, the type of mulch material was significantly ($F_{(5,120)}$ = 5.928, $P$<0.05) varied on the abundance of natural enemies as well as pest abundance ($F_{(5,120)}$ =3.897, $P$ <0.05).

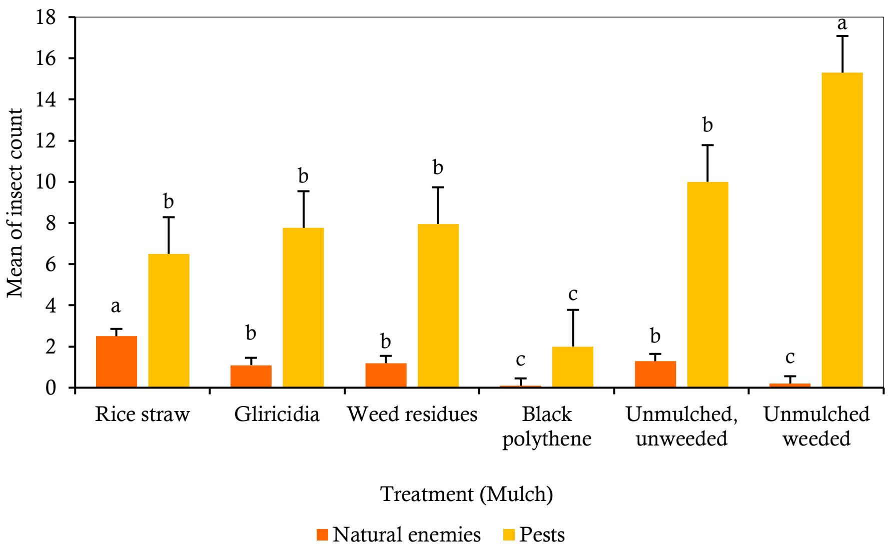


**Figure 3:** Mean of insect count of total natural enemies and pests under different mulch treatments, collected with all sampling methods over a period of two months. Different letters within the same mulch treatment are significantly ($P$<0.05) different according to the Duncan's New Multiple Range Test (DNMRT).

As shown in Figure 3, the highest abundance of pests was found in unmulched weeded plots. There was a significant ($P$<0.05) difference in pest abundance between plots with weeds and without weeds. However, the same in unmulched, unweeded plots were not significantly ($P$>0.05) different from that of rice-straw, gliricidia, and weed residues mulches. The lowest number of pests was recorded in black polythene mulched plots. Due to the heating of soil, as an effect of the polythene mulch, it makes a less favorable habitat for insects' survival (Bharatee *et al.*, 2021).

Of the pests (number of pests; 1070), the highest abundance was found with aphids (81.2%), followed by leafhoppers (7.9%), treehoppers (5.9%), flea beetles (4.3%), and darkling beetles (0.7%).

As shown in Figure 4, aphids ($F_{(5,120)}$=4.857, $P$<0.05), leafhoppers ($F_{(5,120)}$=5.496, $P$<0.05), flea beetles ($F_{(5,120)}$=2.244, $P$<0.05) and darkling beetles ($F_{(5,120)}$=3.814, $P$<0.05) were significantly affected by mulch treatments. However, treehoppers ($F_{(5,120)}$=5.496, $P$>0.05) were not significantly affected by treatments.

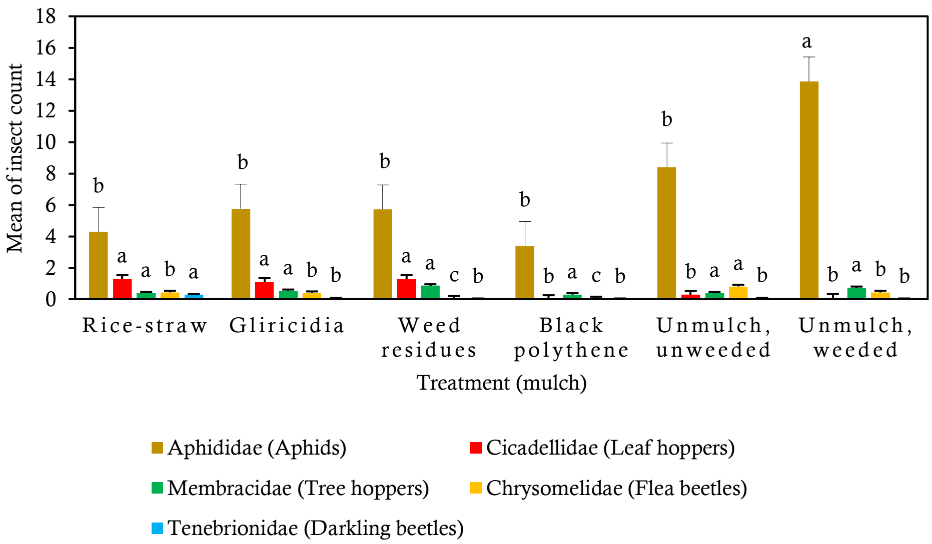


**Figure 4:** Mean of insect count (pests) under different treatments collected with all sampling methods over a period of two months. Different letters within the same mulch treatment are significantly different ($P$<0.05) according to the Duncan's New Multiple Range Test (DNMRT). The same letters on the bars indicates no significant differences ($P$>0.05) among the mean number of natural enemies and pests within the same mulch.

Of the total of natural enemies (number of natural enemies 127), the highest abundance was on spiders (44.1%), followed by coccinellids (18.9%), carabid beetles (17.3%), rove beetles (11.0%), and earwigs (9.5%). As shown in Figure 5, spiders ($F_{(5,120)}$=2.738, $P$<0.05), rove beetles ($F_{(5,120)}$=0.609, $P$<0.05), earwigs ($F_{(5,120)}$=6.066, $P$<0.05), and Coccinellids ($F_{(5,120)}$=8.186, $P$<0.05) were significantly affected by treatments while there was no any significant variation of carabid beetles ($F_{(5,120)}$=1.918, $P$>0.05) between treatments (Figure 5). Twentytwo (22) ground beetles (Family Carabidae) belonging to eight genera were identified as follows: 7 *Bembidion* spp., 2 *Chalaenius* spp., 2 *Drypta* sp., 1 *Goniotropis* spp., 1 *Ophionea* spp. 6 *Pheropsophus* spp., 2 *Scarites* spp., and 1 *Stenolophus* spp. Also, four different species of Coccinellid beetles were identified from the study, such as *Coccinella transversalis*, *Coccinella octomaculata*, *Micraspis discolor* and *Anegleis cardoni*.

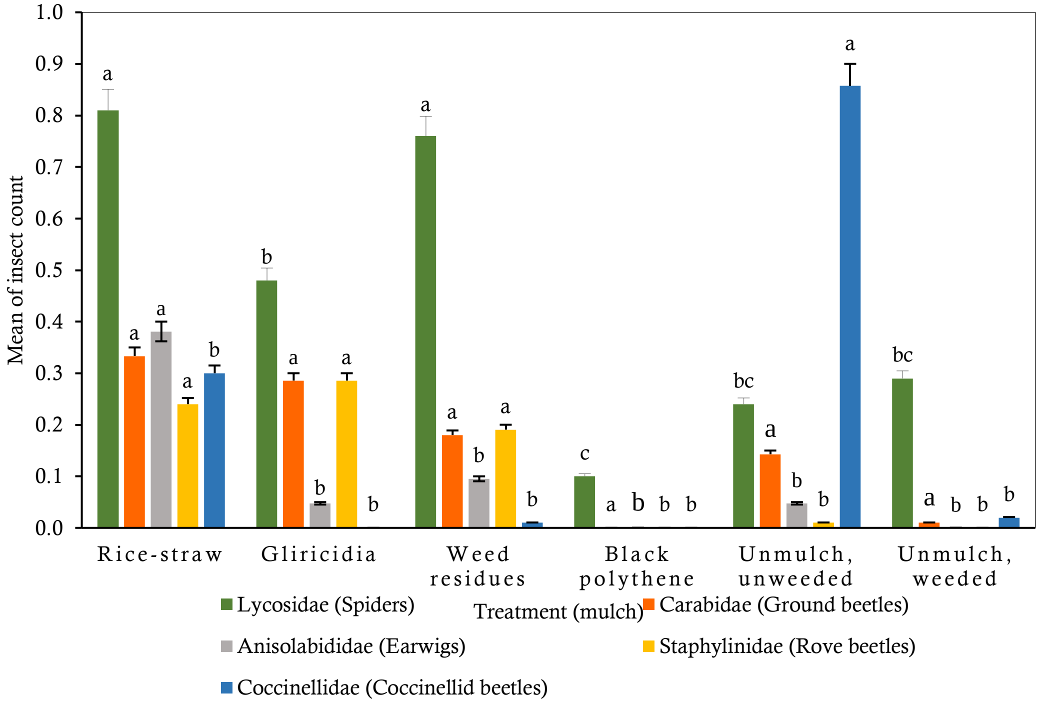


**Figure 5:** Mean count of natural enemies (insects) under different treatments, collected with all sampling methods over a period of two months. Different letters within the same mulch treatment are significantly ($P$<0.05) different according to the Duncan's New Multiple Range Test (DNMRT). The same letters on the bars indicates no significant differences ($P$>0.05) among the mean number of natural enemies and pests within the same mulch.

The highest abundance of natural enemies was found in rice straw mulch plots (Figure 5). There was no any significant ($P$>0.05) difference among gliricidia, weed residues, and unmulched unweeded plots. The lowest number of natural enemies was recorded in plots with black polythene mulch; however, it was not significantly ($P$>0.05) different from unmulched, weeded plots. There was a significant ($P$<0.05) difference in the number between rice-straw mulch and black polythene mulch. A higher abundance of natural enemies in rice straw mulch may be due to the provision of better habitat for natural enemies by providing good ground cover and favorable microclimate for the existence of insects (Johnson *et al.*, 2004). Heating soils that have been covered by polythene mulch leads to the low number of natural enemies reported in polythene mulch treatment may be attributed to the generation of toxic compounds in vapor and liquid phases in the soil with heating by sunlight and accumulates under the plastic mulch, resulting in toxicity against soil flora and fauna as shown by Gamliel *et al.* (2000).

**Assessment of population dynamics of natural enemies and pests over time after mulching**

As shown in Figure 6, the number of spiders increased after the second week of the experiment and the highest number was captured in the third week of the experiment. There was a significant difference in the number of rice straw, gliricidia, and weed residue mulches in the third week compared to the rest of the period ($X^2$ =7.6 df=2 $P$<0.05). This may be due to the existence of a favorable environment that provides suitable shelter sites microclimates for the survival of spiders after mulch application (Latif *et al.*, 2008); however, the numbers were lower in the fourth week, may be due to receiving of heavy rainfall, thereby the movement of spiders was interrupted and as Manns *et al*. (2008) point out some lower activities of them could be observed under heavy showers because the spiders prefer to live on dry surfaces normally.

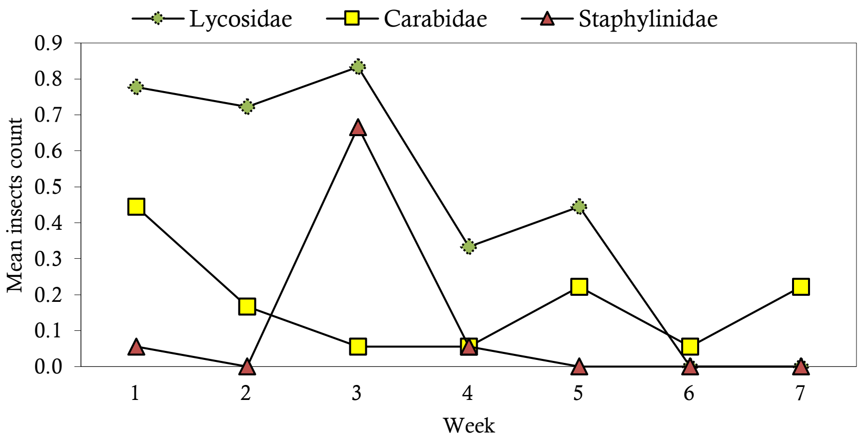


**Figure 6:** The mean insect count of the Family Lycosidae, Carabidae, Staphylinidae at weekly interval.

Both Family Anisolabididae and Family Tenebrionidae had a pattern somewhat similar to Family Lycosidae (Figure 7). As for many invertebrate species, earwigs typically prefer to live in shady, moist conditions to prevent the desiccation of their eggs and nymphs. Their distribution is strongly associated with semi-arid conditions, rainfall, and temperature (Hill *et al.*, 2019). After the second week of the experiment number of earwigs increased. It may be due to having better habitats to shelter after the application of mulches (Brown and Tworkoski, 2004). A relatively lower number of earwigs was reported in the fourth and fifth weeks of the experiments, and this may be attributed to the somewhat sticky nature of soil tending to accumulate water after rainfall. As such, soil becomes less favorable for the existence of earwigs, as reported by Moerkens *et al.* (2012). After the fifth week, the earwigs' population was again increased.

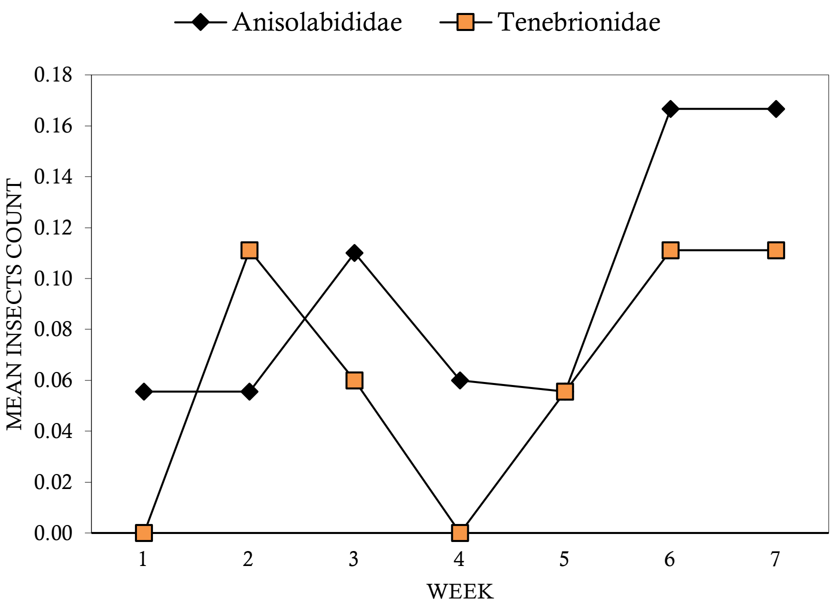


**Figure 7:** The mean insect count of the Family Anisolabididae, Tenebrionidae at weekly interval.

As shown in Figure 7, darkling beetles have also shown some similar patterns to Family Anisolabididae. Their population was increased after mulch application and got reduced in the fourth week as darkling beetles are decomposers; they prefer to live in the open, dry environmental conditions where organic matter is available (Arunraj *et al.*, 2017). The highest number of darkling beetles was recorded in rice straw mulch (Figure 4). The number of darkling beetles increased gradually due to the decaying of mulches, resulting in availability of more organic matter for them (Bartholomew and El Moghrabi, 2018).

The number of leafhoppers (Family Cicadellidae) was high at the outset, but it has reduced after the fourth week and again has increased in the last week of the experiment (Figure 8). There was a significant difference between treatments over the experimental period ($X^2$ =28.75 df=5 $P$<0.05). There were no enough natural enemies at the initial stages resulting in higher amounts of leafhoppers. But, at the latter stages, the leafhopper population has reduced, and this may be due to the development of some natural enemies and having heavy rainfall, and their mobility has been interrupted (Shrestha, 2019). In the seventh week, the leafhoppers population has increased. It may be due to the plant growth in unmulched plots was somewhat lower than that of mulched plots, and the well-grown plants in the latter stages of the experiment provided better habitats for leafhoppers to shelter. However, there was no significant difference among treatments in the seventh week ($X^2$ =3.00 df =2 $P$>0.05).

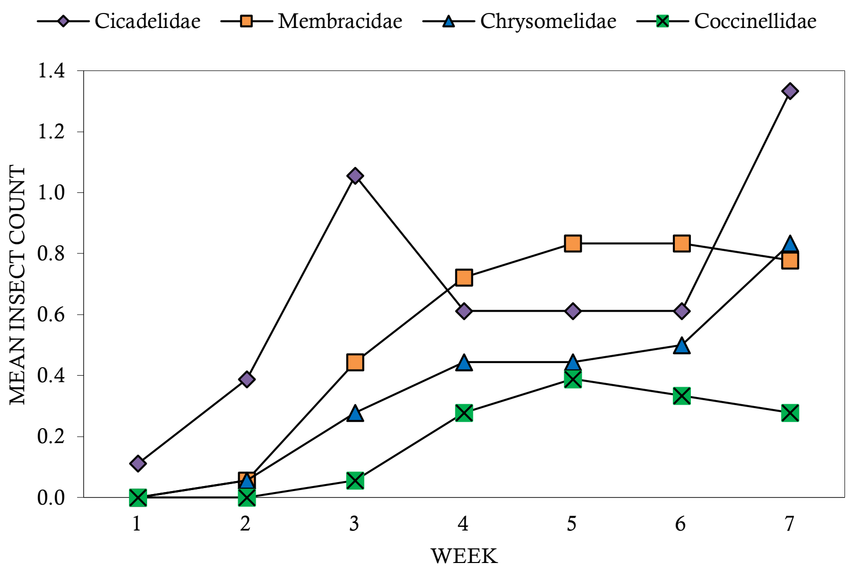


**Figure 8.** The mean insect count of the Family Cicadellidae, Membracidae, Chrysomelidae, Coccinellidae at weekly interval.

In this experiment, four different species of coccinellid beetles were identified (Figure 2). The Coccinellid beetles' population has increased with the increasing aphid population (Figure 9). It may be due to the availability of aphids as the diets of coccinellid beetles for them to survive. However, it is expected to reduce the aphid population simultaneously with the increasing coccinellid beetle population (Farooq *et al.*, 2018). Usually, some aphids reproduce sexually, and some reproduce asexually, and some aphids increase their population using both reproduction methods, depending on their environment (Simon *et al.*, 2011). Therefore, the aphid population increased faster than the development of natural enemies like Coccinellid beetles (Kundoo and Khan, 2017). As this experiment was conducted within a short period, there was an insufficient time to multiply the natural enemy population. It may be the reason for the gradual increase of pests with time, as there were not many natural enemies like Coccinellid beetles.

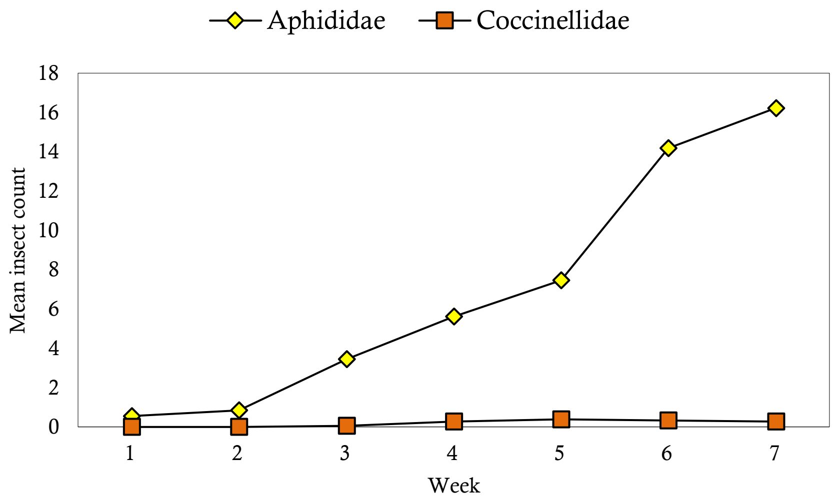


**Figure 9:** The mean insect count of the Family Aphididae, Coccinellidae at weekly interval.

**Assessment of species diversity under different types of mulches**

Table 2 shows species diversity and evenness in different treatments.

**Table 2.** Assessment of species diversity under different types of mulches.

| Mulch type | Shannon-Weaver diversity index (H') | Evenness |
|---|---|---|
| Rice straw | 1.5 | 0.68 |
| Gliricidia | 1.2 | 0.55 |
| Weed residues | 1.6 | 0.73 |
| Black polythene | 0.7 | 0.50 |
| Unmulched,unweeded | 1.2 | 0.50 |
| Unmulched, weeded | 0.9 | 0.46 |

The total species diversity (both natural enemies and pests) was high in weed residues, mulch followed by rice straw mulch, gliricidia mulch, unmulched, unweeded plots, unmulched weeded plots, black polythene mulch. Weed residues mulch had a greater number of species present, but the individuals in weed residue mulch were distributed more equitably among these species than under other mulches. The lowest species diversity (natural enemies and pests) and evenness were recorded in black polythene mulch compared to other mulches. The lowest species richness may be due to heating soils that have been covered with polythene mulch and leading to the production of toxic compounds in vapor and liquid phases in the soil and becoming an unfavorable habitat for soil fauna due to the accumulation of toxic compounds under the plastic mulch (Gamliel *et al.*, 2000). Conversely, high H' values (Table 2) in mulched plots reflect that the mulching makes favorable habitat for inhabiting insects because, The mulches conserve soil moisture, enhance the nutrients status of soil, modify physical characteristics, and provide better shelter sites for the survival of insects (Shirgure *et al.*, 2003).

## CONCLUSIONS

Mulching with dead plant material is found to be creating a more favourable environment for natural enemies of pests in the brinjal crop. Among these, rice straw is found to be a more promising material to use as a mulch in brinjal cultivation to enhance the populations of natural enemies of insect pests, thereby subsequent reduction of the pest populations. The use of black polythene as a mulch reduced abundance of pests in brinjal cultivation. The presence of weeds in brinjal plots also causes to enhance the natural enemy abundance.

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