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Contents

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Some physical and biochemical compositions of the sweet cherry (*Prunus avium* L.) fruit

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Abstract. Given the fact, that the sweet cherry is enjoying growing interest from larger growers, the question is how to diversify the production. Besides producing fruits for fresh consumption, it is necessary to establish new ones, targeted to specific directions for industrial processing. Our research in Maros County has the purpose to select the suitable cherry cultivars for industrial use. The selection examined the fruits which are suitable for industrial processing, has high soluble solids content, dark red colour, can be used as natural food colouring matter. Also, the fruits have excellent quality and high nutritional content value.

Keywords: fruit quality, soluble solids, total acidity, colour intensity, elites

1 Introduction

The cherry fruit grows in small quantities worldwide. Even so, in the temperate zone the cherry plays an important role because of the special character of its fruit, ripening period and its wide utilisation in the food industry [7, 10, 11].

According to FAO dates, the world sweet cherry production ranges between 1.3 – 1.5 million tonnes, in abundant years exceeds 1.6 million tonnes (FAO-STAT, 2008). Regarding the growing area, Europe has a leading role, with more than 50% from the world production. Romania's cherry production in 2000 was cultivated on 7150 acres. In the period between 2010 and 2015 the growing area is estimated to be 67000 acres [3]. The sweet cherry cultivation is determined by the climate, the area, the cultivars and the costs of production (labour costs, technology, market structure). While the Western European countries show a descending production because of their higher labour costs, in countries like Turkey, Spain, South-America and USA the production is growing. The former Soviet Union countries have become important cherry suppliers.

The sweet cherry has become a more sought produce on internal and world markets. It would be appropriate to establish new plantations, where beside intensive cultivation the environmentally safe cultivation is also present. Good results can be obtained by introducing cultivars according to market demands. The availability of the cultivars depends on fruits colours, ripening time, physical and biochemical composition. In the present modern nutritional-philosophy, the organic feeding discovers again forgotten or abandoned dishes, nutritional customs [8, 13, 14]. A substantial part of the world's crop is used for fresh consumption, while in the food industry it is one of the main raw materials, mostly preserved, but it is used as frozen fruit and juice as well. The cultivars with black fruit meat and juice are considered the most appreciated material for the industrial processing, because the sweet cherry can also be used as a natural food colorant [15].

2 Materials and Methods

All investigations in this study were realised in Maros County from 2006 to 2009. The sweet cherry population was evaluated with the purpose to select the suitable cherry cultivars for industrial use. The selection examined the fruits which are suitable for industrial processing, has high solids content, dark red colour, can be used as natural food colouring matter. Also, the fruits have excellent quality and high nutritional content value.

During the evaluation 15 valuable selections have been selected: '*M-10*', '*M-11*', '*M-13*', '*M-18*', '*M-21*', '*M-22*', '*M-71*', '*M-104*', '*M-105*', '*M-108*', '*M-114*', '*M-115*', '*M-116*', '*M-117*', '*M-122*'. '*Germesdorf*', '*Boambe de Cotnari*', '*Szomolyai fekete*' and '*Bicskei fekete*' recognised cultivars were used for control.

In the selected elites 5 physical (fruit diameter, fruit meat mass, pit mass, pit mass in percent of fruit mass and fruit meat in percent of fruit mass) and 4 biochemical characteristics (soluble solid content, total acid content, total sugar content and colour intensity) were investigated during full maturity time.

The main physical characteristics of the fruits were observed by collecting 50 fruits from each individual each year, and the following characteristics were measured under laboratory conditions.

The biochemical characteristics were realised in the chemical laboratory of Sapientia University, Faculty of Technical and Human Science. A 50 fruit sample was used to determine soluble solids content, using digital refractometer (ATAGO Palette PR-101). Total acids content was established by titration with 0,1N NaOH, while in order to determine the total sugar content the conversion table edited by International Sugar chemical Corporation (ISC) was used according to the refraction value. The fruit colour intensity was measured with spectrophotometer (JENWAY 6300) at 380 nm wavelength.

Data analysis

For data analysis one-way ANOVA was used for testing differences in the data of fruit diameter, fruit mass, fruit meat, pit mass, pit mass in percent of fruit mass, fruit meat in percent of fruit mass and the soluble solids between elites and cultivars. Analysis of variance was used in the case of normal sample distribution and large sample number. In the case of lower sample numbers Kruskal-Wallis and Mann-Whitney non-parametric probes were used.

Pearson's linear correlation was computed to compare the mean fruit diameter with the fruit mass. The statistical analysis of the results was carried out by using the SPSS 17.0 for WINDOWS and MS Excel software.

3 Results and discussions

Comparative examination of the physical features of fruits

The results of the physical features of fruits (perspective elites and control cultivar) are displayed in Table 1. The most important defining characteristic of sweet cherry is the fruit diameter. The European Union standard regulates the lower size of the class I at 17 mm diameter in case of the sweet cherry, but the market is more demanding. For fresh consumption the 24 mm diameter indicates the lower size of class I [12].

However, at the cultivars for industrial processing the 16-17 mm diameter is appreciated as first class. Similarly to the control cultivar, more than 60% of the perspective elites have a greater diameter than 17 mm. Comparing the fruit diameter of selections with that of the control cultivar did not show any significant differences.

During the harvest period, the fruit mass plays a prominent role as a major influence on the weight of fruit harvested per unit time. It determines the productivity of harvest, and indirectly the production's profitability as well.

For industrial use, the 3-4 gram fruit mass indicates the highest fruit mass category [1]. The average fruit mass of the investigated selections ranged from 3.45 g to 4.63 g. The '*M-18*' selection is the exception, with 2.76 g fruit mass.

Related to the fruit weight, the pit mass varies between 0.21 g and 0.40 g within the selections. This indicator plays an important role mainly in case of cultivars for industrial processing. The lowest pit mass is shown at the '*M-18*' selection, while the highest pit mass is found at '*M-108*', which is nearly twice the weight of the selection mentioned above.

It is worth to express the individual fruit mass as a proportion of pit mass, because it reflects the relation between the two indicators. The lower pit mass has no effect if it's only associated with low fruit mass. Significant difference was observed between the perspective elites of the above mentioned indicators: while '*M-13*' selection shows 10.63% pit mass percent of fruit mass, the '*M-114*' has only 6.62% (Table 1).

Analyzing the fruit indicators, a positive and significant correlation has been found between the mean fruit diameter and the fruit mass of the selections ($R^2 = 0.932$, at $p = 0.01$ level) (Figure 1.). The correlation reveals the fruit meat consistency and succulence, which constitutes an important feature in industrial processing.

The chemical composition of fruits

The suitability of fruits for industrial processing is influenced by some biochemical characteristics, such as soluble solid, the acidity and sugar content degree and concordance, and the colour intensity of the fruit, which is used as natural food colorant. The total soluble solids content significantly influences the fruit flavour, because a significant proportion is created by the different sugars.

Table 1: The physical characteristics of the fruits (2007 and 2008).

Cultivar and Elites	Fruit diameter (mm)	Fruit mass (g)	Fruit meat (g)	Pit mass (g)	Pit mass in percent of fruit mass (%)	Fruit meat in percent of fruit mass (%)
Szomolyai fekete	17.34abc±0.55	4.09a±0.10	3.79a±0.05	0.30bcd±0.05	7.33cd±1.04	92.67cd±1.04
M-10	16.80bc±1.82	3.47ab±0.98	3.21ab±0.88	0.26de±0.10	7.45cd±0.77	92.55cd±0.77
M-11	16.72bc±1.13	3.45ab±0.78	3.21ab±0.63	0.24de0.15	6.85d±2.77	93.15d±2.77
M-13	17.13abc±2.81	3.58ab±0.11	3.20ab±0.16	0.38ab±0.05	10.63a±1.71	89.37a±1.71
M-18	15.79c±0.52	2.76b±0.34	2.55b±1.28	0.21e±0.06	7.59cd±1.29	92.41cd±1.29
M-21	17.03abc±2.39	3.81ab±0.97	3.54ab±0.98	0.27cde±0.01	7.27cd±2.29	92.73cd±2.29
M-22	16.98abc±0.22	3.61ab±0.56	3.29ab±0.52	0.32abcd±0.04	8.79bc±0.22	91.21bc±0.22
M-71	18.19abc±3.99	4.49a±1.93	4.14a±1.80	0.35abc±0.12	7.83cd±0.58	92.17cd±0.58
M-104	18.26abc±2.25	3.86ab±1.34	3.60ab±1.33	0.26cd±0.01	6.95cd±2.20	93.05cd±2.20
M-105	18.84ab±1.81	4.63a±0.51	4.24a±0.48	0.39a±0.02	8.44bcd±0.40	91.56bcd±0.40
M-108	19.40ab±0.65	4.08ab±0.63	3.68ab±0.63	0.40a±0.00	9.85ab±1.54	90.15ab±1.54
M-114	18.04abc±1.12	3.53ab±1.25	3.29ab±1.18	0.23abcde±0.09	6.62abcd±0.01	93.38abcd±0.01
M-115	18.22abc±1.13	4.06ab±0.68	3.78ab±0.63	0.28abcde±0.05	6.90abcd±0.05	93.10abcd±0.05
M-116	18.70ab±3.28	4.39a±1.43	4.09a±1.43	0.3abcde±0.00	6.95abcd±2.25	93.05abcd±2.25
M-117	19.70a±2.23	4.24a±0.73	3.96a±0.91	0.28abcde±0.04	6.75abcd±2.15	93.25abcd±2.15
M-122	18.08abc±1.85	3.88ab±1.45	3.59ab±1.35	0.30abcde±0.09	7.67cd±0.52	92.33cd±0.52

Explanation: *Mean value ± 95% Confidence Interval for Mean; Different letters refer to p<0.05 (ANOVA).*

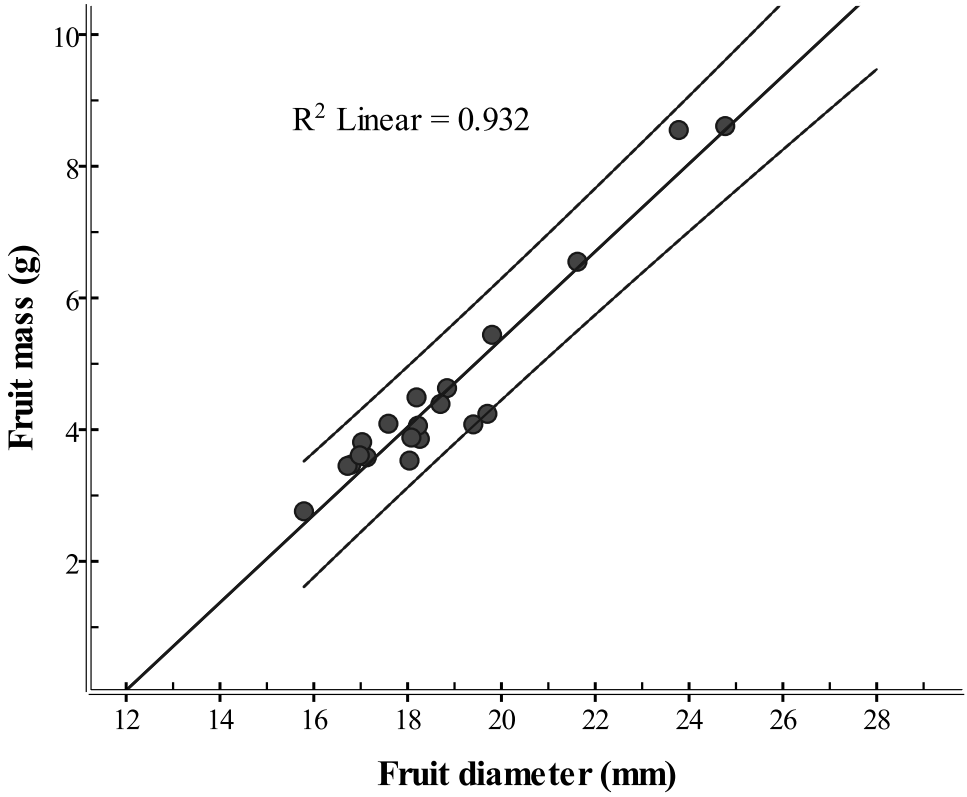

Figure 1: Correlation between the mean fruit diameter and the fruit mass

Total soluble solids

Analyzing the biochemical characteristics of the fruits, the refraction values show the same TSS values at '*M-22*', '*M-18*', '*M-11*' and '*M-13*' selections comparing to nationally recognized cultivars ('*Bicskei fekete*', '*Szomolyai fekete*') exceeding 20 Brix% (Figure 2.). The refraction value of the '*Germersdorf*' cultivars is lower with 7.6-9.1, meanwhile '*Boambe de Cotnari*' shows 11.0-13.0 Brix% differences compared with the TSS values of the above mentioned selections. The difference is statistically significant at $p < 0.001$ level.

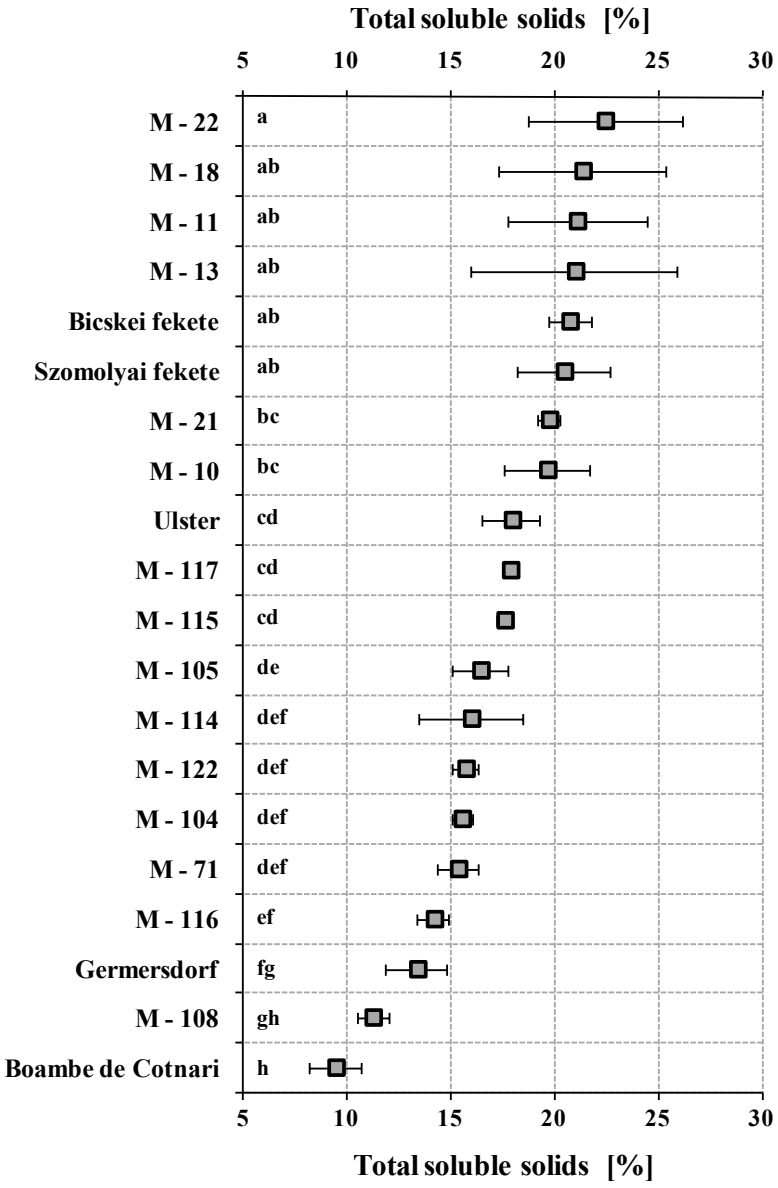

Figure 2: The average TSS concentrations for the period 2007-2009 (Different letters represents significant differences at $p < 0.05$ level (ANOVA)

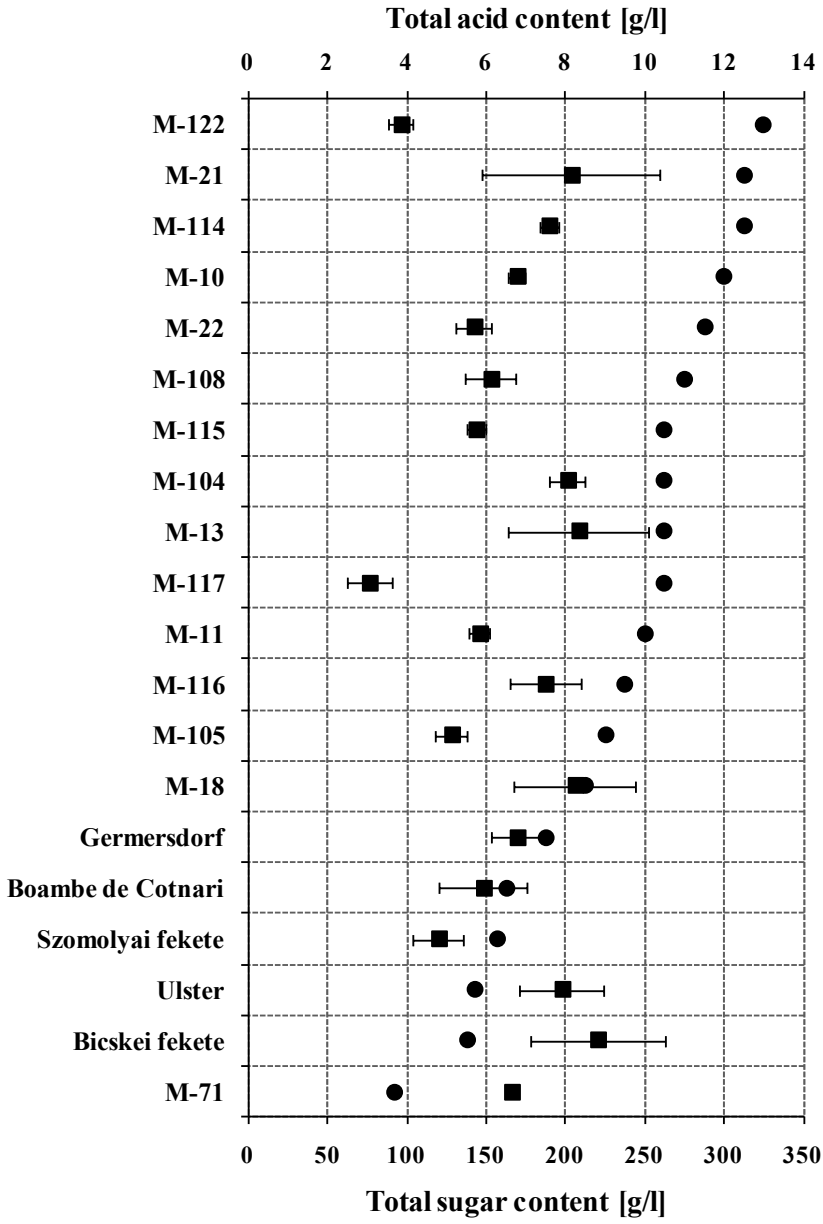

Figure 3: Average total sugar (■) and total acids (•) of fruits (2007 and 2008).

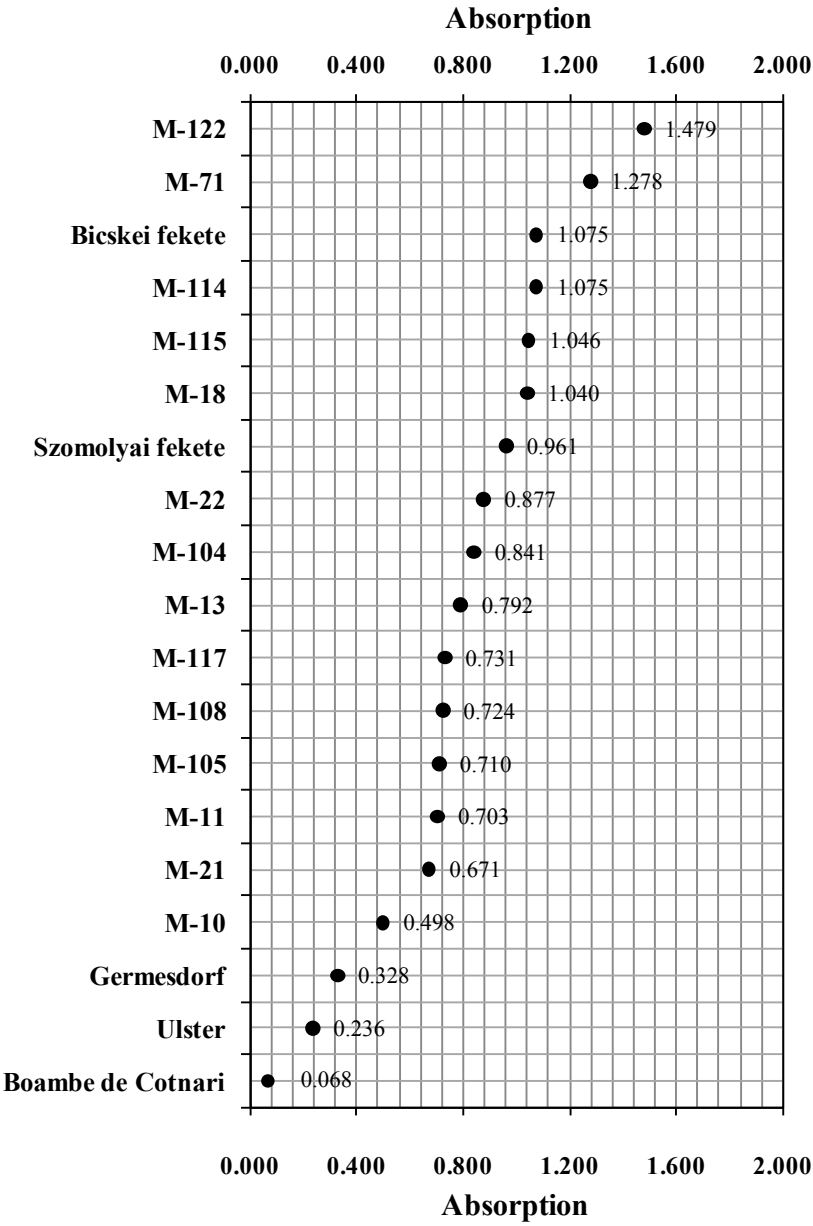

Figure 4: Average absorption spectra of sweet cherry selections and cultivars (2007-2008)

Total acids and sugars content

The investigated prospective elites and control cultivars have very wide total acid content, between 3.7 and 13.0 g/l. Evaluating the values reveals that the control cultivars have low acidity in contrast with the prospective elites (Figure 3.). Referring to sugar content, the '*M-21*', '*M-104*', '*M-13*', '*M-116*' and '*M-18*' selections have higher values compared to the control cultivars.

According to Gyuró and Tóth (1980), Gyuró (1990) and Papp (2003) the flavour of the fruit is greatly influenced by the acid content correlated with the sugar content. Comparing the acid and sugar content concordance of the fruits, the conclusion is that the '*M-104*', '*M-13*', '*M-116*', '*M-18*', '*Germersdorfi*', '*Boambe Cotnari*' and '*Szomolyai fekete*' selections and cultivars shows balanced acid and sugar content. In contrast, the sugar content of the '*M-122*', '*M-21*', '*M-114*', '*M-10*', '*M-22*', '*M-108*', '*M-115*', '*M-117*', '*M-11*' selections is much lower than their values of acidity. At the '*Ulster*', '*Bicskei fekete*' and '*M-71*' selections and cultivars the sugar content is higher than the acidity content.

The results of spectrophotometric evaluation of the fruits

According to literature reports, the '*Bicskei fekete*' and '*Szomolyai fekete*' have intense colorant juice [2, 6, 12]. Their spectrophotometric evaluation of colour intensity at 380 wavelength (nm) ranged from 0.96 to 1.08 absorbance characteristic value. Comparing the absorbance value of the '*M-122*' and '*M-71*' selections with that of the '*Bicskei fekete*', 0.400 and 0.200 difference was observed in favour of the selections (Figure 4.). In case of three selections ('*M-114*', '*M-115*', '*M-18*') higher absorbance values were found than at the '*Szomolyai fekete*' cultivar. Thus, the above selections ('*M-122*', '*M-71*', '*M-114*', '*M-115*', '*M-18*') have more intense pigments than '*Bicskei fekete*' or '*Szomolyai fekete*' nationally recognized cultivars for industrial processing.

4 Conclusion

Based on the results of the experiments, the following conclusion can be made: Between 2006 and 2009, several sweet cherry prospective elites were selected in Mures County with the purpose to determine the suitable ones for industrial processing. The suitable selections are characterized by black shiny fruits, with firm meat, high TSS concentration, intense colorant juice, balanced flavour, sometimes with a slightly sourly taste. The most predominant fruit

dimension is the medium one, but the '*M-117*' and '*M-108*' selections have a fruit diameter between 18 and 21 mm. In the case of fruit for industrial processing, the 18-21 mm diameter defines high quality [3].

The '*M-22*', '*M-18*', '*M-11*' and '*M-13*' prospective elites have solid contents that exceed the amount found in the *Bicske Fekete'* and '*Szomolyai Fekete*' cultivars.

The sugar content calculated on basis of soluble solid content, for '*M-21*', '*M-104*', '*M-13*', '*M-116*' and '*M-18*' shows equivalent values to those found in the '*Bicskei fekete*', making it possible to obtain natural preparations without added sugar.

Spectrophotometric analysis shows that most selections have intense colorant juice. The colour intensity of the juice is accentuated especially at the '*M-122*' and '*M-71*' selections.

These selections could play an important role in a possible breeding program of sweet cherry to expand the present assortment. Therefore a long term national breeding program would be necessary for conserving and improving these individuals.

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The effect of some environment factors on the growth of sweet pepper

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Abstract. The forced white sweet peppers for stuffing are important export items in Hungary. Increasing market requirements need variety specific technologies. Therefore, it is important to gather detailed information of the environmental factors influencing pepper growth to obtain high quality and quantity without neglecting profit. In our trials the effect of some environmental factors were studied on pepper berry growth. Measurements were performed in a heated plastic tent with the white pepper variety Hó in a soilless culture. Data were registered by Phytomonitor.

It could be stated that:

- berry growth depends decisively on light intensity and plant temperature
- berry growth varies between 0,2 mm/day and 2,4 mm/day
- berry growth in greatly influenced by the maximal and minimal plant temperature and trends.

Data can help us to approach optimal environmental conditions in order to obtain higher quality and yield.

Keywords: global radiation, plant temperature, berry growth

1 Introduction

Vegetable forcing is one of the most intensive branches of horticulture. In this way, in all conditions of growing technology it is possible to provide the plant with the most optimistic growing conditions. The optimal temperature for sweet pepper preconditions other ideal environment factors. Such factors are very infrequent in the forcing periods. Light is the most important environment factor, but we cannot influence it economically. Therefore we have to adjust other factors to the all-time light conditions.

The growth of plants is determined by several environment factors. Heat, light, water and nutriments are the most influential. Light and temperature are crucial factors. If one of the environment factor changes others should be changed accordingly in order to avoid worse crop quality [4]. To achieve the best growth and yield it is important to adjust other environment factors to all-time light conditions. Sweet pepper is sensitive to the lack of light, the crop is poorly set, the vegetation is longer, bloom and harvest are getting later [3]. If light is stronger than that it should be, set is fewer and berries are less developed, nodes are short and the crop is low and distorted [4]. Sweet pepper is a C_3 plant and the intensity of photosynthesis is increased only up to a certain intensity of light. The maximum berry growth (impregnation) happens at 300-400 W/m^2 light intensity [1, 2].

During our research the main goals were as fellows:

- to determine optimal plant temperature in different forcing periods in regard to radiation so that the growth of berries would be ideal.
- to determine the extreme high temperature in a certain period where berry growth stops
- to find the optimal night temperature where the intensity of growth is optimal

2 Materials and Methods

Light is one of the environmental factors that can be least influenced by the growers. Due to this, plant temperature was studied in accordance to light together with the tendency of berry development. Phytomonitor was used for our observations. By the use of this instrument stress situations in forcing can be revealed right after their appearance. Numeric values can be traced continuously. Growers can use these data to solve actual problems.

We carried out our research in temperature controlled green house, in hydroponics. The variety applied was Hó F_1.

The instrument can measure and register environment factors (global radiation, air temperature, soil temperature and soil moisture) and plant parameters (stem expansion, berry growth, plant temperature, water cross flow in the stem. We studied sweet pepper berry growth during different phases of forcing.

3 Results and discussions

We looked at the measured data in different intensity of radiation and wanted to see how much is the berry growth in accordance with plant temperature. The below figures (1, 2, 3) show some examples.

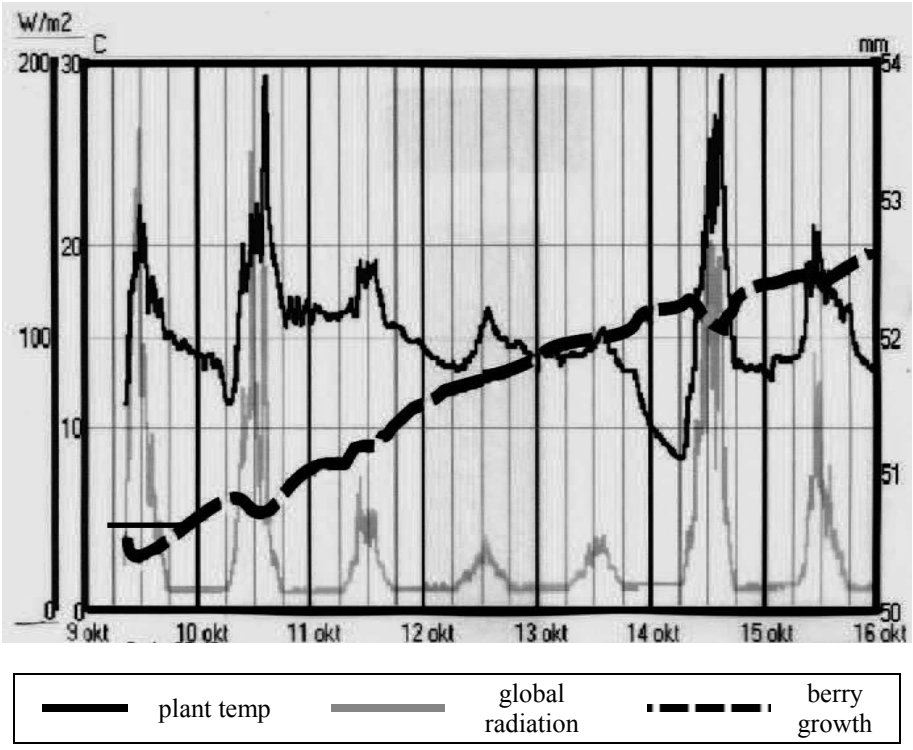

Figure 1: Change of sweet pepper plant temperature and berry growth in 100–200 W/m^2 radiation phase

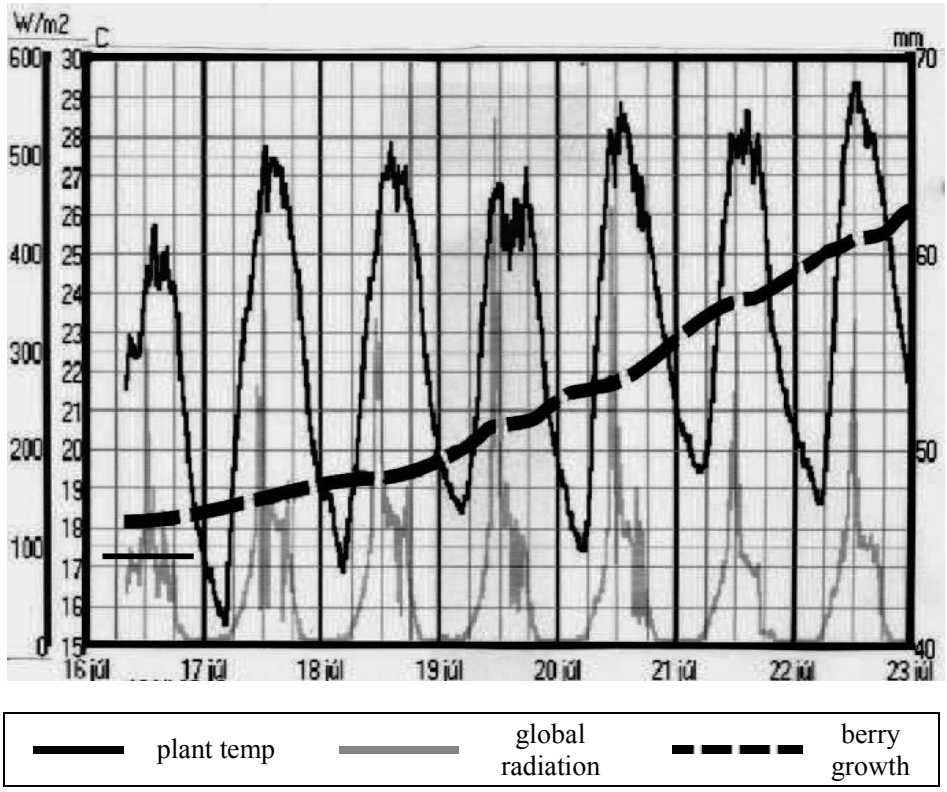

Figure 2: Change of sweet pepper plant temperature and berry growth in 200–300 W/m^2 radiation phase

Based on the data we obtained berry growth was calculated which grower will have in certain radiation intensity in optimal and less optimal conditions (Table 1.).

According to the table we can conclude that radiation intensity and plant temperature largely effect the growth of berries. In low light periods with low radiation index (max. 100 W/m^2) and at 18–20 °C plant temperature berry development is ideal. If plant temperature is higher berry growth is less is optimal at this temperature intensive. Above 400 W/m^2 radiation intensity the optimal plant temperature for optimal berry growth is 24–26 °C. If plant temperature is higher the berry growth will be less.

Table 1: Interrelation of berry growth and plant temperature at different radiation intensity

radiation intensity W/m²	**berry growth mm/day**		**plant temperature °C**
0–100	high	0,44	< 18–20
	low	0,127	> 20
100–200	high	0,867	< 20–21
	low	0,308	> 21
200–300	high	2,37	< 22–23
	low	0,277	> 23
300–400	high	2,09	< 24–25
	low	0,02	> 25
above 400	high	1,92	< 25–26
	low	0,03	> 27

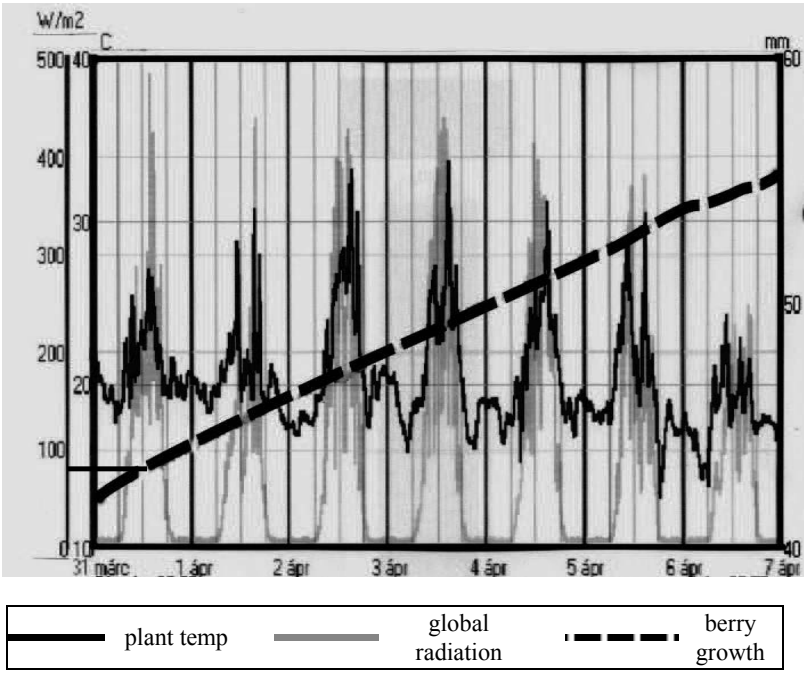

Figure 3: Change of sweet pepper plant temperature and berry growth in 400–500 W/m^2 radiation phase

4 Conclusion and suggestions

Based on our research we can conclude the followings:

- daily rate of berry development grows up to 400 W/m^2 radiation.
- berry growth mostly depends on light intensity and plant temperature
- the rate of berry growth is 0,4–2,37 mm/day depending on the intensity of radiation and optimal plant temperature
- berry growth is also influenced by the maximum and minimum plant temperature and the type of the change.

Considering the above results we can provide optimal environmental factors which result a better quality and bigger crop. The economic advantage of these findings is that growers can adjust the heating to the light conditions. In this way they can save energy and insure the optimal conditions for berry growth.

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Ear properties of direct seeded sweet corn

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Abstract. In our trial we compared the effect of propagation time and floating cover on the growing season on some valuable properties of sweet corn. The following technological variations were compared with the help of the variety Spirit (normal sweet, very early ripening): 1. direct seeded plants with floating cover (with 2 sowing dates); 2. direct seeded plants without cover (with 2 sowing dates). The covering and earlier sowing time had favourable influence on ear weight, and ear length. The combination of earlier seeding time and floating cover results 8 days earlier harvest as compared to the traditional technology (P4).

Keywords: growing season, earliness, covering

1 Introduction

Based on its present growing area, the sweet corn is the vegetable which is grown on the greatest area in Hungary. After dates of Hungarian Fruit & Vegetable Interprofessional Organization in 2003 the growing area was about 38,000 hectares. After 2003 followed a sudden and sharp decline, so in 2005 the growing area was "just" 24,000 hectares. After diminishing, the plant returned in rise, in 2006 against over 30,000 hectares.

As early as in the beginning of the 20th century some researchers [3] highlighted the importance of the sowing date. Ripening can occur earlier when sowing earlier and using high quality seeds as compared to normal or late sowing. [6] and [12], after their multi-year sowing date trial, concluded the following: in the case of an earlier sowing seed germination will be more protracted, but from the point of view of fruit maturing it was more favourable than late sowing.

Also [7], [8] were studied occur of maize generative phenophases. They concluded, that by earlier sowing germination will be more protracted, but silking and harvesting occur sooner than by lately sowing time. After multi-year trial studying the effect of different sowing times on maize development authors concluded the following: a 3 weeks lately sowing time delay one week occur of silking time [2].

Several techniques are known in the art for the purpose of early fresh market shipments: seedling growing or direct seeding with temporary plant cover [11], [4]. Direct seeded sweet corn under vlies cover showed earlier ripening and gave better yields in the experiments of [9]. The plots under vlies cover reached harvest maturity 12 days earlier as compared to the plots with no cover. In case of direct seeding, as propagation method, another earliness increasing solution is the temporary covering with plastic or vlies, used in different combinations. This method reaches about 7–10 days earliness [5]. About the covered early sowing as a technological variation [1] mentioned, that from an early sowed crop, made in first week of April, arranged in twin rows (42 cm) and covered by plastic, we could harvested marketable cobs by the fourth of July.

2 Materials and Methods

The experiment was set up in 2008 on an area equipped for irrigation at the Experimental Farm of the Faculty of Horticulture of the Corvinus University of Budapest. The results of the analysis of the soil sample collected at the beginning of 2006 from the trial area prior to direct seeding are contained in Table 1.

The pH of soil was considered calcareous. The nutrient content of soil in nitrogen was low, in phosphorus very good and in potash good. The test variety was Spirit, a normal sweet corn with a very early growing period (85 days). Average plant height is 159 cm, ear height is 37 cm. Average ear length was 19.6 cm in the variety comparison trials carried out by the Central Agricultural Office and average ear weight was 245 g [10].

Table 1: Soil analysis results

pH_{H2O}	Salt %	Humus %	K_A	P_2O_5 mg/kg	K_2O mg/kg	$CaCO_2$
8,03	0,035	1,31	< 30	293	205	< 1

The following treatments were applied during the experiment:

P1 = uncovered direct seeded (April 8$^{\text{th}}$)

P2 = covered direct seeded (April 8$^{\text{th}}$)

P3 = covered direct seeded (April 21$^{\text{th}}$)

P4 = the control, uncovered direct seeded (April 21$^{\text{th}}$)

By both sowing times (April 8$^{\text{th}}$ and April 21$^{\text{th}}$) a part of the stand was covered with Novagryl floating row cover having a weight of 19 g/m^2 at the two propagation times in order to enhance earliness. The floating row cover was removed on May 13$^{\text{th}}$. The stand was created to contain 60,607 plants per hectare, according to the recommendations of the owner of the variety, at a spacing of $110 + 40 \times 22$ cm in twin rows. Each plot had an area of 6×7m (8 parallel rows and 30 seeds sown in each row). The edge was the outer two rows of the 8 rows of the plot, respectively. Number of replications: 4.

Fertilization was done by top dressing with $\mathbb{N}$. No farmyard manure was applied.

During the experiment, we studied plant growth rates and recorded the time of the occurrence of the major phenological stages. For this purpose, we carried out regular observations (every 3 to 5 days) according to the following:

- appearance of tassels (in 50% of the plants),
- beginning of tasseling (pollen shed has begun on the axes of tassels),
- 50% silking (silks have reached a length of 2 cm on half of the ears),
- "milky stage" (harvest).

During harvest the ears, together with the husks, were collected from the four central (two twin) rows. After that 20 ears of average appearance were selected from each row and the following measurements were carried out:

- unhusked ear weight (gram),
- total ear length (cm),
- depth of seeds (mm).

The statistical analysis of the results was carried out by using the programme RopStat 1.1. When the standard deviations were identical the mean values were compared by pairs using the Tukey-Kramer test, while in the case of the non identical standard deviations the means were compared using the Games-Howell test [13].

3 Results and discussions

The occurrence of the different phenological stages is illustrated by Table 2:

Table 2: Day of occurrence of generative phenophases (day of direct seeding or transplanting = 0)

Treatments	Appearance of tassels	Beginning tasseling	50% female flowering	Milky stage
P1 (IV. 8.)	57. day (VI.5)	64. day (VI.12)	71. day (VI.19)	86. day (VII.3)
P2 (IV. 8.)	54. day (VI.2)	57. day (VI.5)	64. day (VI.12)	84. day (VII.1)
P3 (IV. 21)	45. day (VI.5)	55. day (VI.16)	58. day (VI.19)	77. day (VII.7)
P4 (IV. 21) Control	49. day (VI.9)	59. day (VI.19)	61. day (VI.22)	79. day (VII.9)

The growing season, expressed in days, of the treatment P4 (control) which was propagated according to the existing production practice, was earlier with 6 days compared to the data recorded by the National Institute for Agricultural Quality Control. In our case meant that the beginning of harvest was delayed by 8 days, when compared to the treatment P2 and by 2 respectively 6 days compared to the treatments P3 and P1.

The unhusked ear weight, one of the major yield parameters, is illustrated in Figure 1.

Analysing the measured data for unhusked ear yield, we saw that the average weight of the ears of the treatment P2 (earlier seeded, covered plants) was significantly (at $p<0.01$ level) higher as compared to the other treatments.

The average unhusked ear weight of the P3 treatment (later seeded, covered plants) was significantly higher (at $p<0.01$ level) compared to the uncovered treatment P1 and higher, but not significantly, compared to P4 treatment.

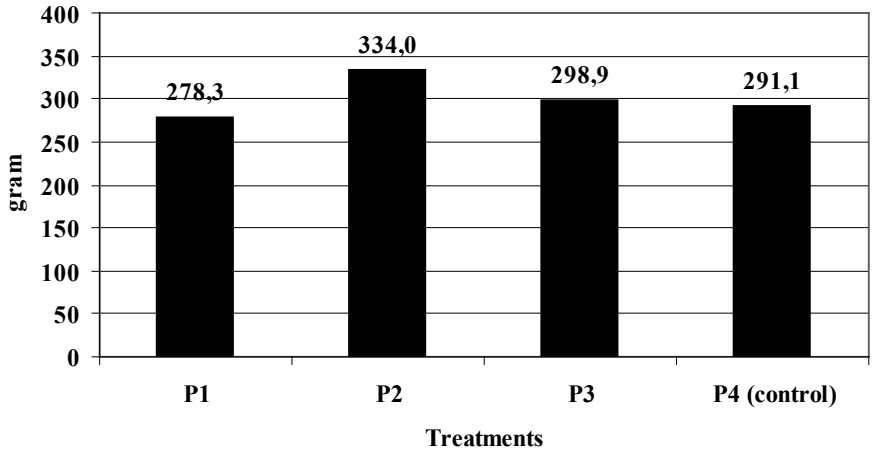

Figure 1: Unhusked ear weight

The data concerning, an important characteristics for market appeal (total ear length) are contained in Figure 2.

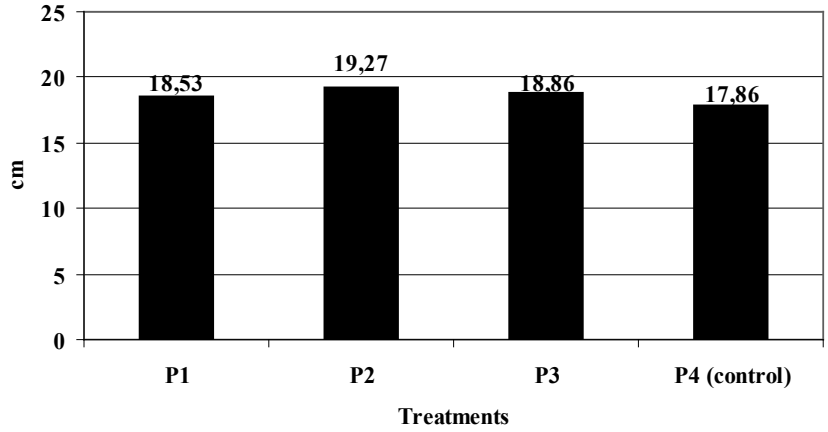

Figure 2: Total ear length

Studying the data of total ear length, we found that the lengths of the later seeded, uncovered (control) treatment P4 were also statistically significantly (at $p<0.01$ level) lower to the sizes of the other treatments (P1, P2 and P3).

The average total ear length of the P2 treatment (earlier seeded, covered plants) was significantly higher (at $p<0.01$ level) compared to the other treatments.

No statistically demonstrable difference was found between the ear length of the treatments P1 and P3.

From customer viewpoint depth of seeds is an important parameter and the measured average results are presented on Figure 3.

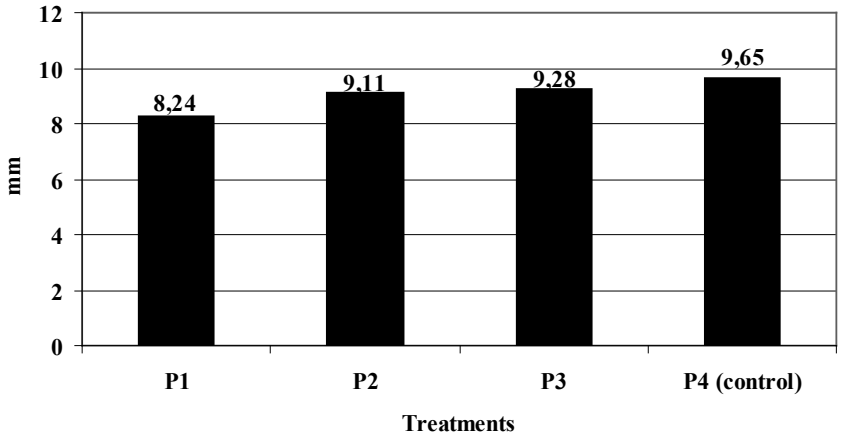

Figure 3: Depth of seed

Analyzing the size (depth) of seeds we observed a statistically demonstrable (at $p < 0.01$ level) difference among control treatment (P4) and other treatments. Seeds depth of earlier seeded, uncovered treatment (P1) was smaller, sustained statistically (at $p < 0.01$ level), compared to the other treatments.

4 Conclusions

The technology with earlier time, direct seeding and floating row cover (P2), in the case of the variety Spirit, resulted 8 days earliness in the total growing period, compared to the uncovered, control treatment (P4), in 2008.

The technology with commonly used time, direct seeding and floating row cover, resulted 2 days earliness in the total growing period, compared to the uncovered, control (P4) treatment, in 2008.

The unhusked ear weight presented the highest results in case of treatment P2 (earlier seeded, covered). In case of later seeded treatments (P3, P4) the results were quite square.

Measuring length of seeds, we observed the same tendency as in case of ear weight. P2 treatment's ear produced the greatest results. From customer viewpoint important parameter, depth of seeds, the later seeded, uncovered treatment P4 presented the better results, in 2008.

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Effects of the Zeolitic Tuff on the physical characteristics of Haplic Luvisol and the quality of fruits on apple orchards

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Abstract. An experiment with the volcanic zeolite tuff of Mirşid (Romania) on a slightly haplic luvisol of an orchard was performed, in order to improve its physical properties as well as to investigate if any influence on the nutrition of the plants (Jonathan apple trees) could be observed. The grinded zeolite tuff (granulation of 1-3 mm) was applied before planting, being incorporated at a depth of 20-25 cm under furrow in the following dosages: 0-30-45-60 t/ha. During the four years of experiment the water-stable structural aggregates in the upper 20 cm layer increased by 24%. The zeolite volcanic tuff had a favourable effect on the N maintenance and brought about the increase of mobile K content in the soil by 2-3 times. It had also a favourable effect on the main nutrients (N,P,K,Ca) in leaves and fruits, inducing a light increase of Fe in leaves and a higher one in the fruit (34%). A moderate increase of sugars, acids and vitamin C in fruits was also noticed. The fruits showed a better storage capacity during the winter period.

Keywords: water-stable aggregates, quality of fruits, storage capacity

1 Introduction

A frequent problem in the intensive orchards situated on heavy, fine-textured soils is the unfavourable physical state of the soil, which starts with the deterioration of its structure. The initial favourable crumb or the granular structure becomes angular blocky, which damages the air and water regime of the soil, causing water stagnation from time to time. As a consequence, the plants' water supply, release and assimilation of nutrients are hindered.

There are several possibilities to prevent the above mentioned phenomenon. One of these is to increase the amount of water-stable aggregates of the soil, in other words the stabilization of the soil structure. Many types of structure stabilizer chemicals – mainly polyacrilates – are known to yield good results. As our aim is to prevent the addition of new chemicals to the soils of the orchard – the soils have already been overloaded by chemicals –, we are looking for an environmentally neutral, easily accessible mineral substance; this should be found abundantly in the nature and our country, having as many favourable influences to the physical state of the soil and to the quality of fruits as possible.

A naturally occurring chemical complying with the above requirements is the volcanic zeolite tuff of Mirşid (Northeastern part of Romania). Our choice was not accidental. Based on the relevant literature the Japanese were doing soil melioration by adding zeolite tuff as early as the 19th century [1, 2, 3]. Several zeolite products have been used in Hungary and Bulgaria to improve the structure, texture, air and the water regime, reaction of the soils, as well as to stimulate the plant [1, 4, 5]. The best Rhine, Tokaj and Eger wines, the well-known Georgian tea, the high quality tobacco from the Rodope Mountains, and the best sweet-smelling roses of Tundzsa Valley derive from soils developed on volcanic zeolite tuffs [6].

Although Romania has important reserves of volcanic zeolite tuff, their agricultural use is insignificant. Aurelia Lăpuşan and co-workers (1974) [7]. have studied the effect of zeolite tuff on clover yield, Ruxandra Bogaci et al. experienced a better absorption of potassium and magnesium by oats and Dactylis glomerata in the Perşani Mountains [3] while Calancea et al. report about the favourable effects of zeolite tuff on potato and maize yield [8].

2 Materials and Methods

Our experiment was performed on a pseudogleyed haplic luvisol of an apple orchard in order to improve its physical properties as well as to investigate if any favorable influences on the nutrition of the Jonathan apple trees could be

obtained.

The upper 50 cm of the soil profile is characterized by the following parameters:

- pH_{H2O} 5.6
- humus 1.49%
- CEC 16.9 me/100g
- PAL 10.5 ppm
- KAL 166.0 ppm
- clay ($\phi < 0.002$mm) 26,5%.

It has to be noted that the clay content increases with depth, to a horizon with pellic character, where the maximum clay content is 50%.

The moderate acid reaction of the soil shows low calcium content, which together with a moderate humus content, independently of any human influence, give an explanation on the unfavourable state of the soil structure.

The soil was amended by grinded volcanic zeolite tuff (granulation of 1-3 mm) originating from Mirşid, near Zalău, having a zeolite content of 74%.

The zeolites represent a specific group of the hydrated aluminium silicate, containing Ca, K, Mg, Fe and Na. Due to their high cation-exchange capacity they are able to adsorb and continuously release water, other molecules and certain cations. Consequently, zeolites may be used for soil improvement.

The percentile chemical composition of the zeolite we used was: Loss of ignition (L.I.) 8.12; SiO_2 69.65; Al_2O_3 12.58; CaO 3.52; K_2O 3.10; Fe_2O_3 1.18; Na_2O 0.80; MgO 0.52; TiO_2 0.29. It was also containing negligible quantities of trace elements.

Location of the experiment and treatment

The experiment was performed at a Jonathan/MM106 apple orchard near Târgu Mureş, with a planting density of 4×2 m. Four treatment variants of the soil with zeolite tuff were performed in four repetitions as follows:

- no treatment (control)
- 30 t/ha
- 45 t/ha 60 t/ha

Scheme of the experiment

1	3	4	2
2	4	3	1
3	1	2	4
4	2	1	3

The grinded zeolite tuff (granulation of 1–3mm) was applied before planting, being incorporated at a depth of 20–25 cm under furrow. Grass (*Lolium perenne*) was seeded in 3 m wide stripes between the rows, and the cut grass was used as cover over 1 m wide bands along the rows.

3 Results and discussions

The effect of the volcanic zeolite tuff on the physical state of the soil was reflected by the improvement of the soil structure, estimated by the increase of the water-stable aggregates. Compared with the untreated soil, at the end of the four year experimental period the zeolite tuff treatment induced a significant increase of the water-stable aggregates in the upper 20 cm of the soil as follows: by 7.5–15.5% in the upper 10 cm and by 13–24% in the layer between 10–20 cm (Table 1). Only the 30 t/ha dosing did not result in a significant increase of the water-stable aggregates in the upper 10 cm layer.

Table 1: Quantitative change of soil's water-stable aggregates of the soil due to the zeolite volcanic tuff

Handling	Depth			
	0–10 cm		10–20 cm	
	Watter-stable-%	Difference	Watter-stable-%	Difference
0t/ha (control)	26,5	–	17,8	
30 t/ha	28,5	+ 1,7	22,2	+ 4,2***
45 t/ha	29,9	+ 3,4**	20,1	+ 2,3***
60 t/ha	30,6	+ 4,1***	22,1	+ 4,3***

$^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$

The lower increase in all four variants of the water-stable aggregates in the upper 10 cm of the soil is the consequence of lower amounts of grinded zeolite tuff being mixed with the soil during the 20–25 cm deep ploughing on the one hand, and on the other hand the higher compaction rate of the soil and the structural damages caused by agricultural equipment.

The importance of the water-stable aggregates consists in the fact that they are less influenced of heavy rains and they are not easily decomposed in their elemental structural particles. As a result, the water stagnation is diminished, and the air and water regime of the soil becomes better.

The volcanic zeolite tuff proved to have a favourable effect on the nitrogen maintenance (an increase of 27–40%) and brought about the increase of the mobile potassium content in the soil by 2–3 times, as well as a moderate increase of the majority of other analyzed nutrients. It also influenced positively the main nutrient content (nitrogen, phosphorus, potassium, calcium) in leaves and fruits, inducing a light increase of the iron in leaves and a higher increase in the fruits (Table 2).

Table 2: Effect of the zeolite volcanic tuff on mineral composition of leaves and fruit

Treatment	N%	P%	K%	Ca%	Fe_{ppm}	Mn_{ppm}	Zn_{ppm}	Mo_{ppm}
				L E A V E S				
0 t/ha	2,19	0,16	1,69	0,94	119	72	31	0,14
30 t/ha	2,42*	0,22**	1,81***	0,98*	124 ns	101***	36 ns	0,19**
45 t/ha	2,52**	0,22**	1,86***	1,14***	125 ns	92***	44**	0,16 ns
60 t/ha	2,46**	0,20*	1,85***	1,22***	134**	89***	36 ns	0,15 ns
				F R U I T				
0 t/ha	0,15	0,023	0,25	0,012	2,60	0,77	1,79	0,012
30 t/ha	0,20**	0,028*	0,29 ns	0,018*	3,45***	0,87*	1,54**	0,014 ns
45 t/ha	0,20**	0,031**	0,36**	0,017*	2,93**	0,82 ns	1,29***	0,018**
60 t/ha	0,22***	0,029*	0,36**	0,016 ns	3,40***	0,70 ns	1,60*	0,016*

$^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$

It is particularly important to mention the absorption of potassium, calcium and manganese in the leaves and the concentration of iron in the fruits. These have a positive effect on the colouring of the fruits, chlorophyll and proteins formation, resistance against metabolic diseases as well as provide a better storage of the fruits too.

The nutrients concentration in the leaves and fruits in the variants treated with zeolite tuff are equal to or approach the optimum levels as mentioned in the literature (cf. Sass 1980), except for the potassium in the leaves that exceeds the upper optimum limit. Such a high concentration of the potassium in the leaves may be and advantage in dry periods of the year, inasmuch as it diminishes the loss of water of the plants by evapotranspiration.

A moderate increase of carbohydrates, acids, and vitamin C in fruits was also observed (see Table 3) in the 45 and 60 t/ha dosing variants. The sugar/acid ratio was constant in all variants.

Table 3: Effect of the volcanic zeolite tuff on the apple's carbohydrate, acid and C-vitamin content

Handling	Red. sugar		Total sugar		Acidity		C-vitamin		Sugar/ acidity
	g %	δ	g %	δ	g %	δ	g %	δ	
0 t/ha	9,6	12,6	0,87		2,9		14,4		
30 t/ha	9,2	- 0,4 ns	11,5	- 1,1 ns	0,92	+ 0,05 ns	3,5	+ 0,6 ns	12,4
45 t/ha	10,5	+ 0,9 ns	13,5	+ 0,9 ns	1,01	+ 0,14 ns	2,9		13,3
60 t/ha	11,7	+ 2,1 ns	15,1	+ 2,5 ns	1,05	+ 0,17 ns	4,1	+ 1,2 ns	14,3

ns insignificant

The storage capacity of the fruits during the winter period increased significantly by approx. 11-15% in cellars. This is important when the farmers do not dispose of modern cooling equipment.

During the four years of experiment the volcanic zeolite tuff did not show any influence on the growth of the plants.

Table 4: Effect of the zeolite volcanic tuff on the storage of apples

Handling	Healthy fruits from 100 1. April	Difference
0t/ha (control)	69	–
30 t/ha	80	+ 11**
45 t/ha	84	+ 15***
60 t/ha	83	+ 14***

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

4 Conclusion

Our experiment proves unambiguously that volcanic zeolite tuffs can be used to improve the physical condition of the soil with air and water regime deficiency. The improvement of the physical condition of the soil has been evaluated by the significant quantitative increase of the water-stable aggregates in the upper 20 cm. The higher quantity of water-stable aggregates induced

a higher non-capillary porosity and – as a consequence – the air and water regime of the soil became better.

Based on our results, the recommended quantity of zeolite tuff in similar climatic and soil conditions is about 45-50 t/ha, but it may vary according to the quantity of clay. For soils rich in clay the doses may be of 60 t/ha or higher.

The zeolite tuff had a favourable effect on the nitrogen maintenance and caused the increase of mobile potassium content in the soil by about 2–3 times. It also had a favourable effect on the main nutrients (N, P, K, Ca) in the leaves and fruits, inducing a light increase of the iron in leaves and a higher increase in the fruit (34%). A moderate increase of sugars, acids, and vitamin C in fruits was also noticed. The fruits showed a better storage capacity during the winter period.

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The hydro-culture of cut flowers

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Abstract. The importance of hydro-cultural growing is significantly increasing. We have been dealing with the hydro-cultural growing of cut flowers at the Department of Ornamental Plant Growing and Maintenance of Gardens at the College Faculty of Horticulture at Kecskemét College since 1988. We started our experiments by growing carnation in growing establishment without soil then we introduced other species of cut flowers and potted ornamental plants into our research work.

Keywords: hydroculture, carnation, Rose, PU sponge, Grodan, Phytomonitor

1 Introduction

Our aim was to examine the effect of Grodan and PU-sponge media on the growth, the yield of flowers, the diameter of the flowers and the length of the stem concerning the species of carnation 'Pink Castellaro'. In case of comparing the species our aim was to examine the effect on the development

of the plants, the yield and the characteristics of the flowers: the diameter of the flower and the length of the stem.

The Phytomonitor instrument is placed in the French Filclaire greenhouse and we at the Floriculture and Park Maintaining Department measure rose culture parameters in hydroponics. We measure the following factors: air temperature, leaf temperature, radiation, relative humidity of air, stem diameter and soil moisture [1].

Using Phytomonitor data processing make it possible to use nutriments in an optimal level thus apply a low-cost environmentally friendly technology.

The effect of the species on the flower diameter of carnation: Most of the species in the experiment reached or exceeded the parameters of 1st class products determined by the standards, minimum was 7.0 except for the values of 6,91 and 6,96 of 'Candy' and 6,87 and 6,89 of 'Ondina' average yearly flower diameter [1].

The largest flower diameters of the red species were experienced in the case of 'Iury' and 'Rodolfo', from the point of flower diameter these species are worth being involved in hydro-cultural growing. In case of the 'Castellaro' species 'Pink Castellaro' produced significantly larger flowers [2]. Experiments with the species:

- 'Danton' is of high growth, of good yield, with large flowers and long stem
- 'Gigi' is of high growth, of good yield, with large flowers and long stem
- 'Iury' is of high growth, of average yield, with large diameter of flower and long stem
- 'White Castellaro' is of high growth, of good yield, with large diameter of flower and long stem
- 'Pink Castellaro' is of high growth, of excellent yield, with large flower and long stem
- 'Candy' is of average growth, of excellent yield, with average size of flowers, with average long stem
- 'Rimini' is of high growth, of good yield, with large flowers, really long stem
- 'Rodolfo' is of high growth, of excellent yield, really large flowers, really long stem

- 'Ondina' is of average growth, of good yield, with average size of flowers, long stem
- 'Olivia' is of high growth, of excellent yield, with large flowers and long stem

Each of the species in the survey is adequate for hydro-cultural growing [2, 3, 4, 5, 6, 7, 8].

2 Material and methods

We made experiments of hydro-cultural growing of carnation with the following species: 'Danton', 'Gigi', 'Iury', 'White Castellaro', 'Pink Castellaro' and 'Candy', 'Rimini', 'Rodolfo', 'Ondina', 'Olivia'.

The experiments of carnation were carried out by the French Filclair growing establishment, growing was arranged in a closed, circular system. The planting of shoots with roots was arranged by 40 pieces/m^2 at the end of May. We applied PU-sponge as the medium of plantation for the comparative experiments, the length of the growing season was one year. The experiment was carried out by repeating the procedure four times. The supply of nutritional material was made by using complex chemical fertilizer, the pH of the nourishing solution was 5,0–6,5, the conductivity was 2,5–3,5 mS and these parameters were continuously controlled. We measured the quantity of the picked flowers from the beginning of blooming each time. We chose 10–10 of the picked flowers by random choice and measured the characteristics of flower quality: the diameter of the flower and the length of the stem.

A PhyTech company plays a pioneer role in the Phytomonitoring ^{TM} system, it detects the plants remotely. It uses advanced methods, collects and analyses the data derived from wireless communication sensors and innovative software. The main purpose is the detection of early plant stress, optimal growth and quality of product to increase income.

3 Results

The effect of the media on the height of the carnation

In case of the hydro-cultural growing of carnation both the polyurethane-ether sponge and Grodan had a good effect on the growth of the plant, both are adequate as a plantation media but the stock grown in the sponge was higher.

The effect of the media on the yield of the carnation

We managed to reach the average flower yield of 7–9 flowers per stem (Figure 1.) characteristic of the traditional chemo-cultural growing in case of hydro-cultural growing in polyurethane-ether sponge and in Grodan that is both are adequate plantation media for hydro-cultural growing.

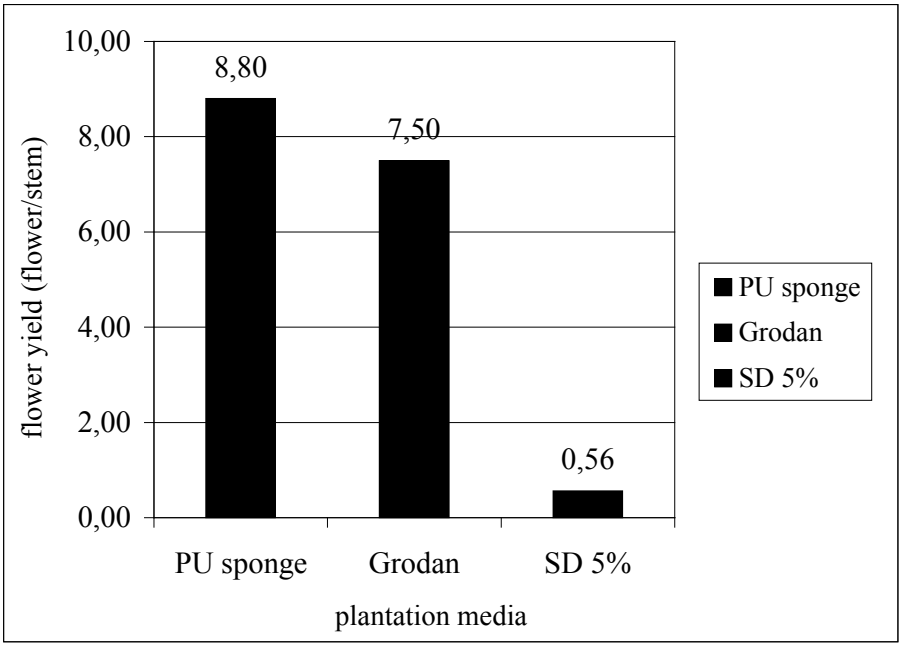

Figure 1: The effect of plantation media on the yearly yield of carnation 'Pink Castellaro' (1999-2000, Kecskemét)

The effect of the media on the flower diameter of the carnation

During the two growing seasons of the experiments the average diameter of the flowers planted in polyurethane-ether sponge and in Grodan reached the parameters of 1st class flowers that is 7-cm flower diameter. We did not experience significantly better results in case of the two media so both are adequate for the hydro-cultural growing of carnation.

The effect of the media on the length of the flower stem of carnation

The plantation media influenced neither the yearly nor the monthly length of the stem significantly in the years of research. Taking the yearly average

into consideration we reached the requirement of 1st class quality that is 55–60 cm stem length in case of both media. Considering all the above both polyurethane-ether sponge and Grodan are adequate media for hydro-cultural growing.

The fluctuation of air temperature well indicates the change of the phases of the day (Figure 2). The expansion of stem follows this cycle. It was pointed out that the higher was the daily maximum temperature the expansion of stems was more intensive. Respectively the fewer daily fluctuation made the stem expansion more stable. By the increase of daily temperature the expansion of stems are significant. The temperature of leaves increases parallel with the air temperature.

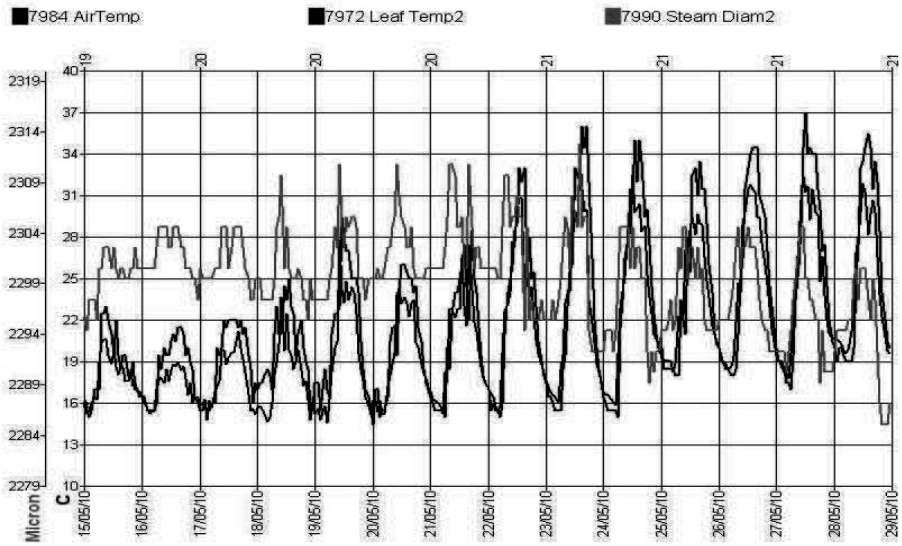

Figure 2: The effect of air temperature on rose leaf temperature and expansion of stem (2010, Kecskemét)

By the increase of temperature the relative humidity decreases. The temperature change of leaves follows the change of air temperature (Figure 3). According to it the relative humidity is higher in the night and lower in the day. The wetness of soil indicates the time of irrigation (Figure 4). The expansion of stems well follows the wetness of the soil.

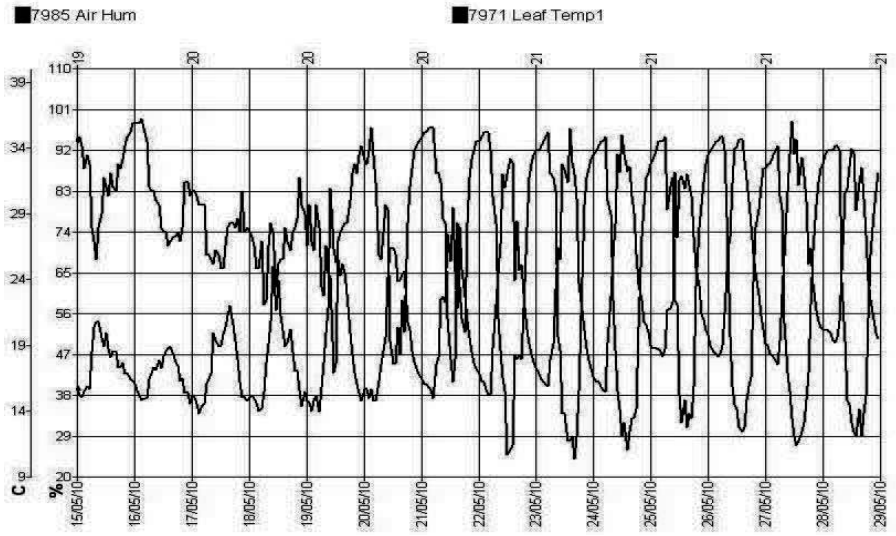

Figure 3: Rose leaf temperature in relation with the air humidity (2010, Kecskemét)

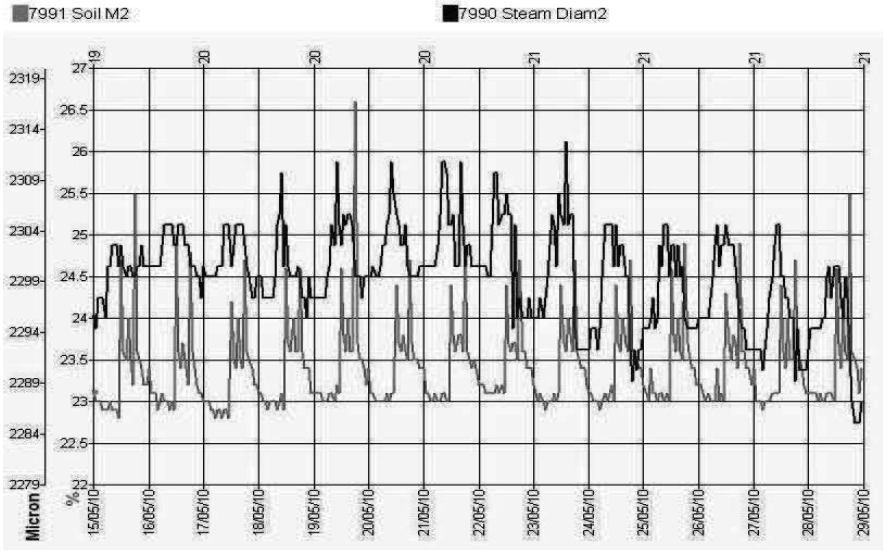

Figure 4: The expansion of rose stem in accordance with soil wetness (2010, Kecskemét)

4 Discussions

Concerning environmental protection PU sponge is more and more adequate media for growing carnation since it can be used until complete decomposition. Both PU sponge and Grodan have got a favourable effect on the growth of the plant, the yield of the flowers and the flower quality characteristics that is why Grodan is also an adequate media for the hydro-cultural growing of carnation. Phytomonitoring is one of the growing decision support devices which gives fast information about the tendency of plant development. It is an information technology which provides the grower with incredibly valuable information about the plant physiologic stage.

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Data to the changes in the structure of shrub layer in a Hungarian oak-forest ecosystem

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Abstract. New type forest decline started in 1979-80 and until now a large-scale decline of oaks appeared which had serious consequences in the structure and function of the shrub layer too. The structural parameters were recorded in the low shrub layer in a 48×48 m "A" plot in 2007 and in 2008. According to our hypothesis, it isn't expected considerably changes of the low shrub species during one year in the number of the individuals, in the proportion of the density and in the average sizes. The dominant shrubs will be remaining those species which could be able to develop policormon.

Keywords: oak forest, low shrubs, number of individuals, size changes, density

1 Introduction

Síkfőkút Project established in 1972 by Prof. Pál Jakucs from KLTE. The results and the data of the model area of a *Quercetum petraeae-cerris* oak forest ecosystem were summarized in the book "Ecology of an oak forest in Hungary. Results of Síkfőkút Project" edited by Pál Jakucs [11]. Within the framework of the "International Biological Program" and of the "Man and Biosphere" research program, complex ecosystem investigations (from 1972) launched already in various research centers abroad [16].

The "Síkfőkút Project" belongs to the Long-Term Ecological Research, that's not easy to spell putting ecological research through long time, but a project methodology with the determinate requirements and terms [23].

The cause of the changes in the forest ecosystem can be identified this decline of sessile oak in the 80s in Hungary [7, 14, 15, 24]. Serious forest declines have been reported since the early 1970's in both Central and Northern Europe and North America [2, 4, 5, 21, 34, 35, etc.]. Many hypotheses have been proposed to explain causes of the forest declines [21, 28, 33, 36]. The effects of anthropogenic factors including acid deposition, oxidants, drought, ozone and heavy metals have been examined most intensively worldwide [1, 38, 40]. There publications are focusing only to the structure, changes and dynamics of forest ecosystem.

The total measurement of shrub layer were followed in the plot of "A" (48 × 48 m) quarter-hectare of 24 hectare total research area every 4-5 years, in the course the researchers analyze the number of species and individuals, density, diversity, shrub sizes, cover of high shrub layer and thereof were doing foliage cover map. On the basis of 7^{th} and 8^{th} measuring on the shrub layer of forest established, that the decease of oaks caused considerably changes in the shrub layer. The serious decline of the oak trees (especially the sessile oak) has been started at 1979/80 in the Síkfőkút site [19, 20]. The results of 2007^{th} measurement from shrub layer were summarized in the paper of MISIK et al. [25].

So far, the surveys did not follow in two consecutive vegetation periods. Therefore, we would like to know, could be demonstrate structural changes less than one year? According to our hypothesis, it isn't expected considerably changes of the low shrub species during one year in the density, in the proportion of density and in the average sizes. The dominant shrubs will be remaining those species in the site which could be able to develop policormon. The following data were suggested important changes, which happened in the structure of oak forest ecosystem.

2 Materials and Methods

The Síkfőkút forest should be in agreement (also at the moment) with the average climazonal Hungarian turkey-oak forest [8, 31, 32]. Some studies can be found at more details from the geographic, climatic, soil conditions and vegetation of the site and the adjacent area descriptions [9, 11, 12, 13, 16, 20, 39].

The survey was done on the "A" plot in the growing season, between 06.06. and 27.06. in 2007 and between 08.07. and 16.07. in 2008, that was designated for structure surveys in 1972 [13]. Firstly, for achieving the most exact results we were studied a shrub level to splitter into low and high layer with the method from 1972 [11, 13, 19, 25]. The researchers were determined the average sizes of shrub species with a "average shrub" random method [16]. We measured the height with a tape-measure and the shoot diameter at 5 cm height above the soil with a slide caliper (to average sizes used random methodology).

The root-studies [17, 18] were confirmed that one part of the shrubs composes policormon (especially *Euonymus verrucosus*, *Euonymus europaeus*, *Ligustrum vulgare* and *Cornus sanguinea*) so the number of shoots above the soil are not the same as the number of individuals [19]. Also, the number of sprout counted by researchers at the measuring, but the number of individuals was used throughout in our study (except a chapter of diversity and evenness).

3 Results and discussions

On the sample area lived 16 and 17 low shrub species in 2007 and in 2008. We were counted together 9495 individuals and 10511 individuals one year later in the "A" quarter-hectare. *E. verrucosus* gave about 56.00% and 48.00% of all the low shrubs in both measuring times, so he was the dominant shrub species.

Following that were *E. europaeus* 10.60% and *Ligustrum vulgare* 10.00% occurence frequency in year 2007. On the next measuring haven't been changed the succession, only the rate of frequency of shrub species. The occurrence frequency of other low species was lower with an order of magnitude. The rate of *Quercus* seedlings (*Q. petraea, Q. cerris* and *Q. pubescens*) were a quite small, referring to hectare it was 5.10% and 5.60% of all low shrubs in 2007 and in 2008, and Q. petraea was the dominant here with rate of 3.90% and 4.10%. It should be mentioned that the oak seedlings showed remarkably fluctuation from year to year. The measured data are summarized in Table 1 and can be found in the study of MISIK et al. [26].

We were making density maps from low shrub layer. Figure 1. shows the density of low shrub layer and shows considerably changes between 2007 and 2008. In the last about three decades the number of *Quercus* seedling showed considerably fluctuation from year to year so we did not consider them on the density map.

Table 1: The number, rate and changes of shrub individuals per low shrub layer

Name of species	Piece/"A" quadrat		Piece/ha		%	
	2007	2008	2007	2008	2007	2008
Acer campestre	544	539	2361	2339	5.73	5.13
Acer tataricum	280	346	1215	1502	2.95	3.29
Cerasus avium	172	178	746	773	1.81	1.69
Cornus mas	117	99	508	430	1.23	0.94
Cornus sanguinea	388	392	1684	1701	4.09	3.73
Crataegus monogyna	155	160	673	694	1.63	1.52
Euonymus europaeus	1006	2034	4366	8828	10.60	19.35
Euonymus verrucosus	5292	4995	22967	21678	55.74	47.52
Juglans regia	15	15	65	65	0.16	0.14
Ligustrum vulgare	953	1104	4136	4791	10.04	10.50
Lonicera xylosteum	22	37	95	161	0.23	0.35
Quercus cerris	42	69	182	299	0.44	0.66
Quercus petraea	370	430	1606	1866	3.90	4.09
Quercus pubescens	109	88	473	382	1.15	0.84
Rhamnus catharticus	14	5	61	22	0.14	0.05
Rosa canina	12	16	52	69	0.12	0.15
Tilia cordata	4	4	17	17	0.04	0.04
Summa	9495	10511	41207	45616	100.0	100.0

The lowest shrubs developed in 2007 in small quadrates "d5", "f2" and "k12" with 4–4 pieces, one year later were found in "m11" and like to 2007 in "f2" the lowest individuals with 4 and 5 pieces. The number of low shrubs was the biggest in "m5", "d1" small quadrate with 289 and 263 individuals, one year later in "d1", "h8" parcel with 364 and 302 individuals. Comparison of the density trends showed that the low shrubs exceeded the 200 pieces limit of individuals only in one small quadrate, but in 2008 were found already 4 small 4×4m quadrates with more than 200 individuals.

The "A" plot shrub density conditions are not necessarily typical for the whole of the forest stand, because the dying *Q. petraea* and *Q. cerris* trees were issued in different sizes foliage gaps on the "A", "B", "C" and "D" quarter-hectare of the sample area [11, 19, 22].

The low shrubs (among 0.50–1.00 m height) of the forest can be divided into shoot, crown and root similarly to the trees. The directly branch is not

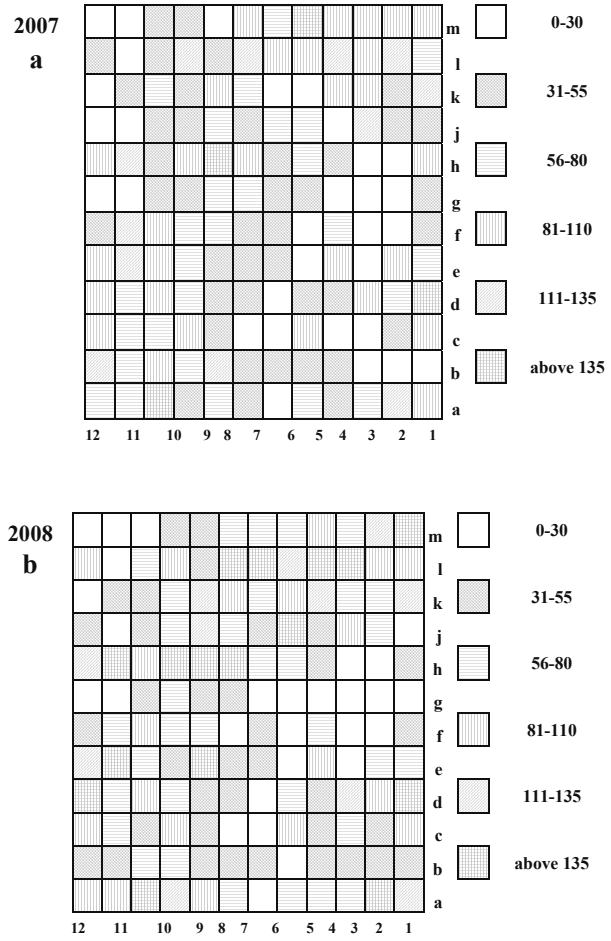

Figure 1: Density of low shrub individuals in small quadrates of the "A" quarter-hectare area in 2007 (a) and in 2008 (b)

typical above the ground surface. Following our measurement of year 2007 and in 2008 the low shrubs were reached 0.32 m and 0.29 m average height. The average trunk (shoot) diameters of low shrub layer were 0.42 cm and 0.35 cm. The biggest low shrub species were based on the average height and shoot diameter *Tilia cordata* and followed them a *Lonicera xylosteum* and *Rhamnus catharticus*. However, these shrub species were presented only small individuals in the plot of research area. The smallest mean values were measured by individuals of *A. campestre* and *E. europaeus* in 2007.

Also in the 2008th survey were reached the greatest average height a 4 specimens of *T. cordata* with 0.53 m, followed them a *L. xylosteum* and *Crataegus monogyna* individuals with 0.52 m and 0.42 m average heights. The researchers were measured the biggest average shoot diameter of "A" sample area by individuals of T. cordata with 0.80 cm, followed them *Cr. monogyna* with 0.64 cm, then *L. xylosteum* with 0.52 cm mean diameter. In point of the dominant shrub species on the sample area have got already other picture. Among of them both in 2007 and in 2008 had got the biggest average sizes a *Cornus mas* and *C. sanguinea*, or rather a *Cr. monogyna*, while *Acer campestre*, *A. tataricum* and *Euonymus sp.* to least were grown.

Only by comparison, on the beginning of the measurements the lower part of the shrub layer below 1 m (low shrub layer) reaches 0.32 m average height. The average trunk diameters of the low shrub layer (the shoot thicknesses) are 0.34 cm [11]. The average sizes are shown in Table 2.

Table 2: Average shrub sizes of the low shrub layer in 2007 and in 2008

Name of species	Height (m)		Shoot diameter (cm)		Measured individuals (pc)	
	2007	2008	2007	2008	2007	2008
Acer campestre	0,16	0,23	0,29	0,34	40	64
Acer tataricum	0,27	0,25	0,33	0,25	32	68
Cerasus avium	0,22	0,20	0,32	0,21	35	24
Cornus mas	0,40	0,40	0,54	0,50	41	32
Cornus sanguinea	0,38	0,40	0,37	0,39	40	66
Crataegus monogyna	0,37	0,42	0,54	0,64	57	60
Euonymus europaeus	0,16	0,16	0,34	0,25	52	118
Euonymus verrucosus	0,29	0,34	0,40	0,40	99	169
Juglans regia	0,33	0,28	0,40	0,23	5	10
Ligustrum vulgare	0,36	0,31	0,49	0,36	52	89
Lonicera xylosteum	0,57	0,52	0,65	0,52	11	16
Quercus cerris	0,15	0,13	0,26	0,19	4	14
Quercus petraea	0,18	0,18	0,30	0,28	60	84
Quercus pubescens	0,15	0,11	0,23	0,19	10	26
Rhamnus catharticus	0,51	0,25	0,50	0,16	4	3
Rosa canina	0,37	0,18	0,32	0,19	7	8
Tilia cordata	0,58	0,53	0,85	0,80	4	4
mean	0,32	0,29	0,42	0,35	32	50

In recent years, some studies have shown that only some Quercus seedlings could be grow above 25 cm height in the site, and the number of seedlings lower from this value decreases from year to year [24].

The biggest average cover value was measured by individuals of *T. cordata* with 1155.00 cm^2 in 2008, followed them *L. xylosteum* with 1119.13 cm^2 foliage cover and *Juglans regia* with 1066.20 cm^2 average cover value. However, these shrub species were lived only with small individuals in the year of research. Among the dominant low shrub species were measured biggest mean cover values by *L. vulgare* and by *C. sanguinea* with 435.27 cm^2 and 611.97 cm^2 parameters. The smallest cover values had got *Q. pubescens* and *E. europaeus* with 80.58 cm^2 and 95.04 cm^2 foliage cover. Among the determining shrub species on the site the individuals of *Acer sp.* and *Euonymus sp.* have got characteristically small cover values. The researchers were measured the total cover value with the cover of all individuals of shrub species of low shrub layer in the sample area ("mean cover values × number of individuals" for each species) and this value was 2668.54 m^2, so in 2008 in the plot of "A" 48×48 m (2304 m^2) quarter hectare were measured 115.82% simplification cover value, this is the area, which covered all living low shrub individuals. These detailed values for the every shrub species in the study area are shown in Table 3.

Finally we were summarized the most important results. On the sample area lived 16 and 17 low shrub species in both measuring with 9495 and 10511 individuals. *E. verrucosus* was the dominant shrub species about 56.00% and 48.00% of all the low shrubs. The rate of *Quercus* was a quite small in every year. The lowest shrubs were found in 2007 in 3 pieces small quadrats with 4–4 individuals, one year later were found 2 quadrats with 4 and 5 individuals. The number of low shrubs was the biggest in 2-2 small quadrat with maximum 289 and 263 individuals, one year later with 364 and 302 individuals. Following our measurement of year 2007 the low shrubs were reached 0.32 m average height. The average trunk (shoot) diameters of low shrub layer were 0.42 cm. One year later this average parameters were decreased with a small-scale. We were measured 502.97 cm^2 mean foliage cover value in 2008. The smallest cover values had got *Q. pubescens* and *E. europaeus*.

Table 3: Average foliage cover values of the low shrub layer in 2008

Name of species	Foliage cover (cm^2)	Measured individuals (pc)	Total cover (m^2)
Acer campestre	332.71	250	179.33
Acer tataricum	382.35	200	132.28
Cerasus avium	291.30	50	51.85
Cornus mas	758.00	40	75.04
Cornus sanguinea	611.97	200	239.90
Crataegus monogyna	882.94	50	141.26
Euonymus europaeus	95.04	350	19.32
Euonymus verrucosus	236.53	350	1181.32
Juglans regia	1066.20	10	15.99
Ligustrum vulgare	435.27	350	480.57
Lonicera xylosteum	1119.13	15	41.41
Quercus cerris	235.05	20	16.22
Quercus petraea	187.00	200	80.41
Quercus pubescens	80.58	50	0.71
Rhamnus catharticus	235.80	5	1.18
Rosa canina	445.60	5	7.13
Tilia cordata	1155.00	4	4.62
mean	502.97	126	156.97

4 Conclusion

The research involved studies on the potential causes of the sessile oak decline, which was reported throughout Hungary (climate change, acidic rain, soil conditions changes, toxic elements etc.) [7, 10, 15]. This procession is not incomparable in the world. Different studies report the decline of various oak forests, the causes of mortality and not least the take placed processing in the forest ecosystem [3, 6, 27, 29, 30, 37, 41]. We are focusing to the cause of the the low shrub layer changes in the forest ecosystem after the decline of sessile oak. The results suggest the following main conclusions: the number of species in the "A" plot sample area did not changed less than one year. Those species lived with a significant number of individuals, which could be able to develop policormon, for example *Euonymus sp.*, and *L. vulgare*. The number of *Quercus* seedlings did not changed considerably compared to year 2007. The Quercus seedlings could not appear in the shrub layer and the rate of these seedlings were a quite small. According to our measurements, only

some *Quercus* seedlings (especially *Q. petraea*) were reached or exceed the 30 cm height limit, and the biggest individual was 39.60 cm in 2007.

We could not find important changes in the data of average height and the shoot diameter of species. In contrast, in the number of individuals and the shrub density conditions of the 4×4 m small quadrates of the "A" plot over a period of one year measured already considerably changes.

This is good exemplify that the number of individuals increased by more than 1000 individuals from 2007 to 2008. The *E. europaeus* had got a privileged role in the increasing of the number of individuals.

Moreover, about 60% of the small quadrates shifted into a different density category during one year. This is good exemplifying that 84 of the total of 144 small quadrates (58.33%) shifted into a different density category during one year. In addition, the important realignment of a density conditions is not only a direct consequently of the ascendant number of individuals, because we found some small quadrates, wherein under one year were decreased the number of individuals. Importantly, it was found that in 2008 were increased the number of the quadrates, where more than 111 or 135 low shrub individuals per small quadrates lived.

However in terms of density conditions remarkably realignment was occurred. This is the most important change in the research area between 2007 and 2008. From these data we could draw a far-reaching conclusion, if we analyze in detail the weather conditions of the related years. However, we have no more possibility to analyze more details within the available extension of this paper.

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Biochemical and enzymatic alterations after application of fipronil, thiomethoxam and malathion to *Odontotermes obesus* (Isoptera: Termitidae)

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Abstract. In the present investigation toxic effects of synthetic termitisides, fipronill, thiomethoxam and malathion were determined in Indian white termite *Odontotermes obesus*. Thermites have shown very high toxicity to fipronil, thiamethoxam and malathion. It is proved by very low LD_{50} obtained i.e. 7.75, 9.0 and 11.50 μg/gm respectively. When termites were treated with sub-lethal doses (40% and 80% of LD_{50}) of each pesticide have significantly reduced (significant at $p < 0.05$) the glycogen level (40.2% and 50%) after 16 h in test insects in comparison to control. Similarly pesticides caused significant alterations in the level of certain molecules i.e. free amino acids, lipid protein DNA and RNA. In addition to it each pesticide significantly ($p < 0.05$) increased level of ALP, ACP, LDH, GPT and GOT enzymes while significantly ($p < 0.05$) decreased (72.75% and 73.45%) the level of acetylcholinesterase. It confirms neurotoxic effects of each pesticide.

Keywords: *Odontotermes obesus*, termiticides, fipronill, thiamethoxam, malathion, lipid, protein, glycogen, enzymes

1 Introduction

Termites are highly destructive polyphaguos insect pests, which largely damage plants, agricultural crops as well as stored household products. Both soldiers and workers of termites cause heavy damage to the agriculture crops, wood, fibers, cellulose sheets, clothes and food commodities. Termites heavily infest sugarcane, millet, barley, maize, paddy and vegetable crops. Therefore to control termite infestation in crop field various synthetic pesticides such as chlorodane [1, 2], borate [3], hexaflumuron [4] cypermethrin hydroquinone, fibronil and indoxacarb are used [5]. Fipronil is a highly toxic insecticide belongs to phylpyrazole class [6]. It shows neurotoxicity and disrupts central nervous system. However, toxic effects of synthetic pesticides are well known but very few reports are available on biochemical and enzymatic alterations caused by these termiticides. However, in the present investigation toxic effects of fipronil, thiomethoxam and malathion were determined on certain enzymes and biomolecules. More specifically, biochemical alterations in the level of DNA, RNA, Protein, Lipid, amino acid glycogen and various metabolic enzymes such as acid phospatase (ACP), alkaline phosphatase (ALP), lactic dehydrogenase(LDH), glutamate pyruvate transaminase(GPT), glutamate oxaloacetate transminase(GOT) and acetylcholinestrase (AChE) were measured after fixed time interval of treated and untreated termites.

2 Material and methods

Termites and plant material

Termite, *Odontotermes obesus* (Rambur) both soldier and workers were collected from the University garden and temporary culture was maintained in the laboratory at 37° C ± 2°C at 80% RH by providing green leaves as food material. Termite culture was protected from light illumination, by using black paper sheets wrapped around the glass containers (12 × 9 inch). Insects were provided fresh food material (green grass and cellulose pulp) and it was changed regularly after 24 h.

Toxicity bioassays

For determination of LD_{50} of fipronil, thiomethoxam and malathion, 5 gm of each pesticide was dissolved in 1 L of water separately. For evaluation of insecticidal efficacy of each pesticide serial concentrations i.e. 10 μg, 20 μg, 40

μg, 80 μg, 160 μg, 320 μg, and 640 μg was coated on cellulose paper strip of 1×1 cm^2 in size and air-dried. These pre-coated paper strips were placed in the center of Petri dishes (42 mm diameter) and 1 gm termite workers ($\sim$ 125 in number) were released in each Petri dish to observe the toxic effect of above extracts separately. Insects were exposed up to 24 hrs and number of living and dead termites was recorded after visualizing the their movements. Insects with no apparent mobility and external stimuli were counted as dead. Mortality was recorded after 4, 8, 12 and 16 of treatment and LD_{50} was calculated in μg/gm body weight. Three replicates were set for each control and test.

Determination of bio-molecular parameters

Termite workers (1gm.) were treated with 40% and 80% of LD_{50} of fipronil, thiomethoxam and malathion by spray. Changes in the level of various biomolecules were measured after 4, 8, 12 and 16h. For this purpose, termites were sacrificed, homogenized and centrifuged to prepare whole body extracts for biomolecular estimation. Few important biomolecules such as glycogen, total free amino acids, total lipid, nucleic acids (DNA and RNA) and total protein were determined.

Glycogen

Glycogen contents were measured according to method of Dubois et al. [7]. Glycogen content in unknown samples (supernatant) is calculated by using standard curve drawn with known amount of glucose. The blank was set by taking 0.50 ml of 5% TCA and 6 ml of concentrate H_2SO_4. The amount of glycogen was expressed in gm/100gm of body weight of termites. Three replicates were set to obtain precision and accuracy.

Total free amino acids

Level of free amino acids was determined by using method of Spies et al. [8]. For calculation, standard curve was prepared by using known amount of glycine and is expressed in gm/100gm body weight of termites. Three replicates were used and data is statistically analyzed by ANOVA method.

Total Lipids

Level of total lipids in whole body extracts of termite was estimated according to method Floch et al. [9]. Total lipid contents were weighted at the end

and expressed in gm/100gm body weight of termites. Three replicates were set and data was statistically analyzed by ANOVA method.

Nucleic acids

Level of nucleic acids in whole body extracts of termites was estimated according to method Scheidner et al. [10]. For this purpose 1 gm of termite workers were fed with 40% and 80% of LD_{50} of synthetic pesticides separately. Insects were scarified and homogenized in 5% TCA with glass-glass homogenizer at 15,000 × g for 25 minutes.

DNA

For DNA estimated with diphenylamine reagent and blue colour developed in the solution, which is measured at 595 nm (O.D.).

RNA

For RNA estimated with orcinol reagent green colour was developed, which was measured at 660 nm.

Total protein

Total proteins of termites were estimated according to Lowry et al. [11] method with the help of Folin phenol Ciacalteu reagent. Blue colour, was measured at 600nm. Three replicates were set for each experiment. Standard curve was prepared by using various concentrations of Bovin serum albumin.

In vivo determination of enzymatic parameters

To observe the effect on enzymatic parameters 500 mg of adult termite workers were provided 40% and 80% of LD_{50} of synthetic fractions. Insects were sacrificed at 4 h interval up to 16 h for the measurement of level of various enzymes. Insects were homogenized in phosphate saline buffer (pH 6.9) in a glass-glass homogenizer and centrifuged in cold for 25 minutes at 15,000 × g. Supernatant was isolated in a glass tube and used for the estimation. Three replicates were set for each bioassay.

Determination of acid and alkaline phosphatase

Acid and alkaline phophatase activity in termites was determined according to the method of Bergmeyer [12]. For the determination of acid phosphatase level p-nitrophenyl phosphate sodium salt were used. A yellow colour developed which was measured at 420 nm. Standard curve was prepared by using different concentrations of p-nitrophenol. Enzyme activity was expressed as the amount of p-nitrophenol formed/30 min/mg protein.

Determination of lactic dehydrogenase

Activity of lactic dehydrogease was measured according to the method of Annon [13]. For this purpose, 500 mg of termite workers were provided 40% and 80% of LD_{50} of each pesticides with diet and termites were homogenized in cold PBS and centrifuged to prepare whole body extract. Enzyme activity was expressed as μ moles of pyruvate reduced/ 30 min/mg protein.

Determination of glutamic-pyruvate transaminase (GPT) and glutamic-oxaolacetic transaminase (GOT)

GPT activity in whole body extract of termites was measured according to the method of Reitman and Frankel [14]. For this purpose, treated termites were homogenized in ice cold PBS buffer and estimation was done with the help of α-ketoglutaric acid, KH_2PO_4, 2–4 dinitrophenyl hydrazine solution. The optical density was noted at 505 nm and blank was set with water to make the background absorbance zero. Standard curve was prepared by using oxaloacetic acid as standard. The enzyme activity was expressed in units of glutamic-pyruvate transaminase activity/mg protein.

Determination of acetylcholinesterase

Acetylcholinesterase activity was determined according to the method of Ellman et al. [15]. For estimation of AchE level 0.050 ml of supernatant was mixed with (10 mm path length cuvette) 0.10 ml freshly prepared acetyl cholinethioiodide solution (5×10^{-4} M) and into it 0.05 ml DTNB (0.19818 gm/l) a chromogenic agent and 1.45 ml of PBS (pH 6.9) were added. The change in absorbance was recorded at 412 nm regularly for three minutes at 25°C. Enzyme activity was expressed in m moles 'SH' hydrolyzed per minute per mg protein.

Statistical analysis

The LD_{50} of each pesticide was determined in worker termites by using Probit analysis. Mean, standard deviation, standard error and Student t-test were applied by ANOVA program [16].

3 Results

Biochemical alterations

Fipronil, thiamethoxam and malathion showed 7.75, 9.0 and 11.50 μg/gm LD_{50} against *O. obesus*. 40% and 80% of LD_{50} of fipronil caused significant ($p < 0.05$) decrease in glycogen level at 4 h of treatment i.e. 50.61% and 50.92% in comparison to control. Fipronil also caused significant ($p < 0.05$) decrease in amino acid level 76.67% and 91.57% after 4 h of treatment. A similar dose of fipronil also caused a significant decrease in lipid level after 4 h of treatment i.e. 87.38% and 87.95% in comparison to control. In the same experiment both DNA (74.94% and 84.00%) and RNA (95.00% and 87.14%), levels were found to be decrease after 16 h of treatment. A similar dose of fipronil also caused significant ($p < 0.05$) decrease in protein level after 8 h of treatment in comparison to control i.e. 72.30% and 59.61% respectively (Table 1; Figure 1).

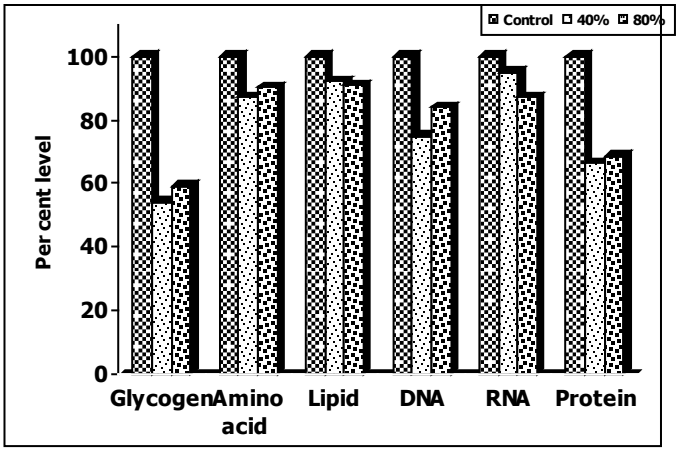

Figure 1: Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of fipronil at 16 hour

Table 1: Effect of 40% and 80% of LD_{50} of fipronil on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.011 (100)	1.64*±0.006 (50.61)	1.66*±0.045 (50.92)	1.36*±0.009 (41.71)	1.47*± 0.013 (45.09)	2.28*±.014 (69.93)	1.65*±.021 (50.61)	1.76*±0.010 (53.98)	1.93*±0.028 (59.20)
Amino acid	0.866±0.003 (100)	0.664*±0.005 (76.67)	0.793*±0.001 (91.57)	0.539*±0.025 (62.24)	0.757*±0.002 (87.41)	1.04*±0.002 (120.09)	0.835*±0.002 (96.42)	0.754*±0.006 (87.06)	0.783*±0.013 (90.41)
Lipid	1.046±0.001 (100)	0.914*±0.002 (87.38)	0.92*±0.001 (87.95)	0.872*±0.00 (83.36)	0.792*±0.008 (75.71)	0.975*±0.002 (93.21)	0.97*±0.014 (92.73)	0.963*±0.001 (92.06)	0.95*±0.003 (90.82)
D.N.A.	0.85±0.028 (100)	0.653*±0.003 (76.82)	0.642*±0.003 (75.52)	0.593*±0.003 (69.76)	0.575*±.0.004 (67.64)	0.685*±0.003 (80.58)	0.727*±0.002 (85.52)	0.637*±0.002 (74.94)	0.714*±0.002 (84.00)
R.N.A.	0.98±0.023 (100)	0.892*±0.003 (91.02)	0.845*±0.002 (86.22)	0.875*±0.006 (89.28)	0.718*±0.001 (73.26)	1.01*±0.001 (103.06)	0.884*±0.001 (90.20)	0.931*±.002 (95.00)	0.854*±.007 (87.14)
Protein	5.2±0.046 (100)	3.65*±0.023 (70.19)	3.38*±0.021 (65.00)	3.76*±0.014 (72.30)	3.10*±0.029 (59.61)	3.66*±0.012 (70.38)	3.52*±0.006 (67.69)	3.45*±0.029 (66.34)	3.56*±0.012 (68.46)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

In a similar experiment when termites were treated with 40% and 80% of LD_{50} of thiamethoxam glycogen level was found to be significantly ($p < 0.05$) decreased after 4 h of treatment i.e. 59.20% and 53.06%. Similar results were obtained in amino acid level. It was found to be decreased when termites treated with 40% and 80% of LD_{50} of thiamethoxam i.e. 62.93% and 86.60% after 4 h of treatment. Similarly, lipid level was also found to be de-creased after 4 h of treatment i.e. 68.16% and 87.66% later it was found to be increase was observed at 12 h of treatment i.e. 65.03% and 93.88% in comparison to control. A similar dose of thiamethoxam also caused significant decrease in DNA and RNA level i.e. 81.17% and 83.17% & 89.38% and 88.97% in comparison to control. Thiamethoxam also caused significant decrease in protein level i.e. 59.80% and 69.80% after 4 h of treatment in comparison to control (Table 2; Figure 2).

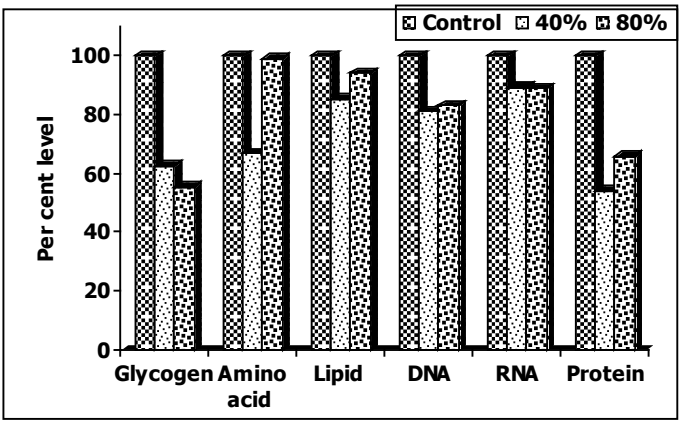

Figure 2: Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of thiamethoxam at 16 hour

When termites were treated with 40% of LD_{50} of malathion, it caused significant decrease in glycogen level i.e. 42.02% and 34.97% after 16 h of treatment in comparison to control (Table 3; Figure 3). While amino acid level was found to be slightly increased i.e. 100.23% after 4 h of treatment with 40% of LD_{50} of malathion. Similarly 80% of LD_{50} of malathion also caused significant ($p < 0.05$) decrease in amino acid level i.e. 87.29% after 16 h treatment in comparison to control. A similar dose of malathion also caused significant ($p < 0.05$) decrease in DNA and RNA level 80.58% and 81.76% & 72.75% and 93.57% after 16 h treatment in comparison to control respectively. Similarly at the same dose protein level was also found decreased up to 41.53% and 22.30% in comparison to control (Table 3; Figure 3).

Table 2: Effect of 40% and 80% of LD_{50} of thiamethoxam on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.0115 (100)	1.93*±0.005 (59.20)	1.73*±0.027 (53.06)	1.67*±0.041 (51.23)	1.54*±0.02 (47.23)	2.12*±0.014 (65.03)	1.99*±0.043 (61.04)	2.04*±0.018 (62.57)	1.81*±0.034 (55.52)
Amino acid	0.866±0.003 (100)	0.545*±0.002 (62.93)	0.75*±0.0023 (86.60)	0.527*±0.002 (60.85)	0.735*±0.005 (84.87)	0.917*±0.003 (105.89)	0.868*±0.0058 (100.23)	0.578*±0.012 (66.74)	0.859*±0.0048 (99.19)
Lipid	1.046±0.001 (100)	0.713*±0.003 (68.16)	0.917*±0.003 (87.66)	0.592*±0.0028 (56.59)	0.822*±0.0058 (78.58)	0.975*±0.009 (93.21)	0.982*±0.0029 (93.88)	0.892*±0.0025 (85.27)	0.985*±0.0035 (94.16)
D.N.A.	0.85±0.028 (100)	0.672*±0.001 (79.06)	0.713*±0.002 (83.88)	0.547*±0.0057 (64.35)	0.624*±0.0037 (73.41)	0.723*±0.009 (85.06)	0.744*±0.003 (87.52)	0.69*±0.004 (81.17)	0.707*±0.0038 (83.17)
R.N.A.	0.98±0.023 (100)	0.79*±0.0042 (80.61)	0.818*±0.005 (83.46)	0.754*±0.0017 (76.93)	0.756*±0.0037 (77.14)	0.913*±0.0012 (93.16)	0.925*±0.0093 (94.38)	0.876*±0.0059 (89.38)	0.872*±0.0025 (88.97)
Protein	5.2±0.046 (100)	3.11*±0.018 (59.80)	3.63*±0.014 (69.80)	3.23*±0.022 (62.12)	3.54*±0.023 (68.07)	2.74*±0.017 (52.69)	3.5*±0.034 (67.30)	2.82*±0.009 (54.23)	3.43*±0.012 (65.96)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

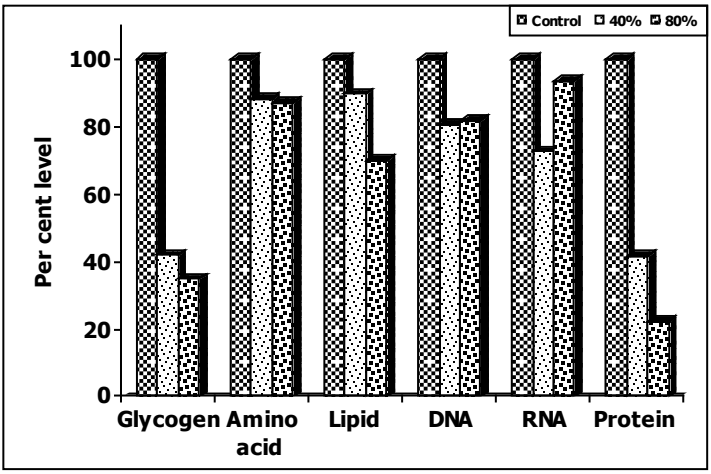

Figure 3: Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of malathion at 16 hour

Enzymatic alterations

In another experiment termites were treated with 40% and 80% of LD_{50} of fipronil, which caused a significant ($p < 0.05$) increase in acid phosphoatase (107.49% & 107.47%), alkaline phosphatase (105.60% & 105.06%), lactic dehydrogenase (103.24% & 102.65%) and glutamate pyruvate transaminase level (106.51% & 106.51%) after 4 h of treatment in comparison to control respectively. Later on decrease was recorded after 16 h of treatment of all the above enzymes. In a similar treatments fipronil ccaused a significant ($p < 0.05$) decrease in glutamate oxaloacetate transaminase (80.91% & 80.03%) and acetyl cholinesterase (60.60% & 61.12%) in termites body in comparison to control respectively (Table 4; Figure 4).

In another experiment termites were treated with 40% and 80% of LD_{50} of thiamethoxam. It caused increase in acid phosphoatase (114.63% & 113.55%), alkaline phosphatase (118.88% & 124.64%), lactic dehydrogenase (105.78% & 104.22%), glutamate pyruvate transaminase (110.83% & 112.62%) and glutamate oxaloacetate transaminase (110.83% & 108.59%) after 4 h of treatment in comparison to control respectively. Though the level of acetyl cholinesterase was also found to be increased at 4 h of treatment i.e. 110.45% and 101.72% respectively but at 16 h treatment a very high decrease was observed i.e. 72.75% and 73.45% in tested termites in comparison to control (Table 5; Figure 5).

Table 3: Effect of 40% and 80% of LD_{50} of malathion on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.0115 (100)	1.85*±0.009 (56.74)	1.76*±0.009 (53.98)	1.74*±0.005 (53.37)	1.38*±0.029 (42.33)	1.56*±0.009 (47.85)	1.66*±0.012 (50.92)	1.37*±0.02 (42.02)	1.14*±0.017 (34.97)
Amino acid	0.866±0.003 (100)	0.868*±0.0087 (100.23)	0.83*±0.002 (95.84)	0.74*±0.0018 (85.45)	0.79*±0.002 (91.22)	0.586*±0.010 (67.66)	0.756*±0.002 (87.29)	0.764*±0.0032 (88.22)	0.756*±0.0021 (87.29)
Lipid	1.046±0.001 (100)	1.12*±0.033 (107.07)	0.933*±0.006 (89.19)	1.09*±0.017 (104.21)	0.891*±0.0012 (85.18)	0.97*±0.003 (92.73)	0.765*±0.0015 (73.13)	0.94*±0.002 (89.86)	0.734*±0.0022 (70.17)
D.N.A.	0.85±0.028 (100)	0.842*±0.0087 (99.05)	0.725*±0.0012 (85.29)	0.779*±0.0026 (91.64)	0.715*±0.0018 (84.12)	0.738*±0.003 (86.82)	0.753*±0.0014 (88.58)	0.685*±0.0028 (80.58)	0.695*±0.0088 (81.76)
R.N.A.	0.98±0.023 (100)	0.92*±0.0027 (93.87)	0.925*±0.0026 (94.38)	0.881*±0.0072 (89.89)	0.927*±0.0058 (94.59)	0.84*±0.004 (85.61)	0.928*±0.0014 (94.69)	0.713*±0.003 (72.75)	0.917*±0.0036 (93.57)
Protein	5.2±0.046 (100)	3.29*±0.023 (63.26)	2.25*±0.017 (43.27)	2.91*±0.041 (55.96)	1.92*±0.041 (36.92)	2.54*±0.04 (48.84)	1.54*±0.04 (29.61)	2.16*±0.015 (41.53)	1.16*±0.015 (22.30)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

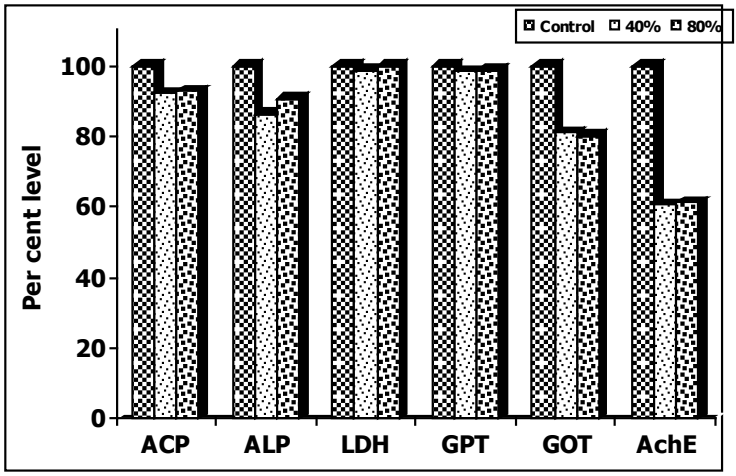

Figure 4: Comparison of acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of fipronil at 16 h

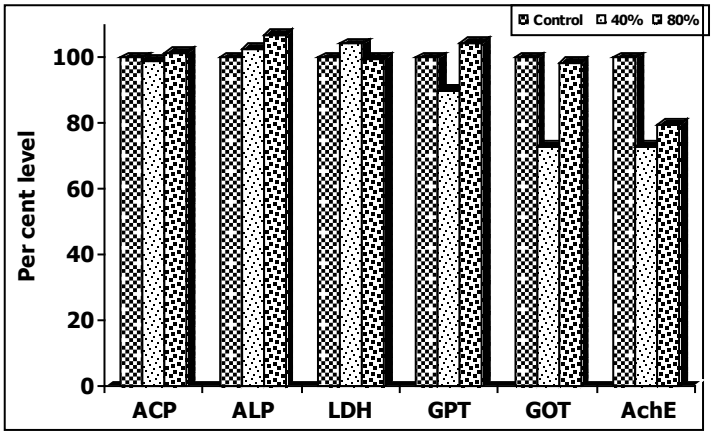

Figure 5: Comparison of acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of thiamethoxam at 16 h

Table 4: Effect of 40% and 80% of LD_{50} of fipronil on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85±0.022 (100.00)	5.21*±0.012 (107.49)	5.21*±0.0088 (107.49)	5.11*±0.014 (105.42)	5.11*±0.015 (105.36)	4.96*±0.0185 (102.19)	4.93*±0.0328 (101.71)	4.47*±0.0188 (92.21)	4.50*±0.0088 (92.71)
ALP	1.25±0.0057 (100.00)	1.32*±0.0058 (105.60)	1.31*±0.015 (105.06)	1.31*±0.0026 (105.14)	1.29*±0.002 (103.22)	1.17*±0.0115 (93.60)	1.28*±0.0017 (102.48)	1.08*±0.0173 (86.40)	1.13*±0.016 (90.40)
LDH	14.59±0.039 (100.00)	15.06*±0.0218 (103.24)	14.97*±0.02 (102.65)	14.96*±0.0058 (102.53)	14.89*±0.013 (102.10)	14.56*±0.0115 (99.79)	14.87*±0.006 (101.91)	14.37*±0.012 (98.51)	14.57*±0.02 (99.90)
GPT	6.55±0.015 (100.00)	6.98*±0.02 (106.51)	6.97*±0.0033 (106.51)	6.76*±0.012 (103.15)	6.88*±0.012 (105.13)	6.66*±0.012 (101.62)	6.73*±0.018 (102.79)	6.41*±0.02 (98.42)	6.46*±0.0088 (98.67)
GOT	1.64±0.023 (100.00)	1.62*±0.01 (98.78)	1.63*±0.0058 (99.39)	1.58*±0.042 (96.21)	1.56*±0.012 (95.32)	1.46*±0.0029 (88.73)	1.42*±0.012 (86.99)	1.33*±0.0065 (80.91)	1.311*±0.002 (80.03)
AchE	0.0116±0.009 (100.00)	0.0114*± 0.00079 (97.98)	0.014*±0.00028 (123.56)	0.0097*±0.00002 (83.64)	0.00893*±0.00012 (77.01)	0.00815*±0.0032 (70.25)	0.0081*±0.0034 (69.45)	0.007*±0.006 (60.60)	0.0071*±0.04 (61.12)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant at ($P < 0.05$, student t-test)

- acid phosphatase (ACP) and alkaline phosphatase (ALP)- µ moles of p-nitrophenol formed /30 minute/mg protein.
- lactic dehydrogenase (LDH): µ moles of pyruvate reduced/ 30min/mg/protein.
- glutamate-Pyruvate transaminase (GPT): Units of glutamate-pyurvate transaminase activity/hour/mg protein.
- glutamate oxalo acetate transaminase (GOT): Units of glutamate oxalo acetate transaminase activity/ hour/ mg protein.
- Acetylcholine esterase (AchE): µ moles 'SH' hydrolysed/min/mg/protein.

Table 5: Effect of 40% and 80% of LD_{50} of thiamethoxam on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85±0.022 (100.00)	5.56*±0.0061 (114.63)	5.51*±0.0048 (113.55)	5.48*±0.0027 (113.09)	5.46*±0.0051 (112.69)	5.10*±0.0059 (105.13)	5.06*±0.026 (104.38)	4.82*±0.004 (99.27)	4.94*±0.017 (101.75)
ALP	1.25±0.0057 (100.00)	1.49*±0.0058 (118.88)	1.56*±0.012 (124.64)	1.45*±0.0152 (116.24)	1.49*±0.0033 (119.46)	1.386*±0.0042 (110.88)	1.42*±0.0047 (113.54)	1.28*±0.024 (102.61)	1.34*±0.0085 (106.96)
LDH	14.59±0.039 (100.00)	15.43*±0.029 (105.78)	15.21*±0.0044 (104.22)	15.23*±0.015 (104.38)	15.03*±0.012 (103.04)	15.19*±0.0088 (104.13)	14.81*±0.028 (101.53)	14.96*±0.0088 (102.58)	14.57*±0.0088 (99.91)
GPT	6.55±0.015 (100.00)	7.26*±0.015 (110.83)	7.37*±0.0088 (112.62)	7.08*±0.029 (108.16)	7.17*±0.012 (109.51)	6.92*±0.035 (105.60)	6.95*±0.01 (106.11)	6.83*±0.01 (104.27)	6.84*±0.02 (104.52)
GOT	1.64±0.023 (100.00)	1.80*±0.0058 (110.83)	1.78*±0.0017 (108.59)	1.70*±0.0012 (103.43)	1.75*±0.0015 (106.89)	1.59*±0.0039 (96.72)	1.66*±0.012 (101.31)	1.47*±0.014 (90.20)	1.61*±0.0018 (98.45)
AchE	0.0116±0.009 (100.00)	$0.0128*\pm7\cdot10^{-4}$ (110.45)	$0.0118*\pm3\cdot10^{-4}$ (101.72)	$0.016*\pm54\cdot10^{-4}$ (138.50)	$0.014*\pm15\cdot10^{-4}$ (124.13)	$0.0098*\pm3\cdot10^{-4}$ (84.83)	$0.0096*\pm2\cdot10^{-4}$ (83.10)	$0.0084*\pm2\cdot10^{-4}$ (72.75)	$0.0092*\pm6\cdot10^{-4}$ (79.45)

Table 6: Effect of 40% and 80% of LD_{50} of malathion on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85±0.022 (100.00)	5.51*±0.0058 (113.60)	5.26*±0.012 (108.45)	5.18*±0.021 (106.80)	5.16*±0.0088 (106.39)	4.96*±0.037 (102.34)	4.99*±0.0088 (102.88)	4.76*±0.0088 (98.21)	4.56*±0.011 (94.02)
ALP	1.25±0.0057 (100.00)	1.42*±0.0047 (113.54)	1.36*±0.0058 (108.80)	1.35*±0.0088 (108.26)	1.34*±0.0037 (106.98)	1.26*±0.0145 (101.33)	1.33*±0.0058 (106.00)	1.13*±0.0088 (100.63)	1.15*±0.017 (92.26)
LDH	14.59±0.039 (100.00)	15.20*±0.031 (104.20)	15.19*±0.038 (104.16)	15.08*±0.043 (103.40)	15.04*±0.0176 (103.12)	14.93*±0.032 (102.33)	14.94*±0.0145 (102.42)	14.68*±0.038 (100.63)	14.5*±0.025 (99.38)
GPT	6.55±0.015 (100.00)	7.05*±0.017 (107.68)	7.04*±0.019 (107.53)	6.96*±0.012 (106.31)	6.94*±0.026 (105.90)	6.73*±0.012 (102.79)	6.73*±0.012 (102.69)	6.56*±0.015 (100.20)	6.53*±0.0218 (99.79)
GOT	1.64±0.023 (100.00)	1.66*±0.012 (101.42)	1.64*±0.0176 (100)	1.61*±0.0037 (98.45)	1.56*±0.0115 (95.12)	1.58*±0.0029 (96.62)	1.48*±0.0066 (90.45)	1.43*±0.015 (87.19)	1.38*±0.012 (82.72)
AchE	0.0116±0.009 (100.00)	0.01*±0.005 (87.06)	0.012*±0.001 (103.45)	0.0092*±0.008 (79.59)	0.00929*±0.000078 (80.08)	0.00826*±0.008 (71.26)	0.0085*±0.007 (73.56)	0.007*±0.001 (60.63)	0.0070*±0.001 (60.05)

In a similar experiment when termites were treated with 40% and 80% of LD_{50} of malathion, it showed a significant ($p < 0.05$) increase in acid phosphoatase (113.60% and 108.45%) and alkaline phosphatase (108.26% & 106.98%) glutamate pyruvate transaminase (107.68% & 101.42%) and glutamate oxaloacetate transaminase (107.53% & 100.00%) after 4 h of treatment in comparison to control (Table 6; Figure 6). Further, a similar dose of malathion caused slight variation in lactic dehydrogenase level i.e. 100.63% and 99.38% in comparison to control respectively (Table 6; Figure 6). Similar increase was also observed in acetyl cholinesterase level after 4 h treatment i.e. 103.45% and 97.98% respectively, but later on drastic decrease was found at 16 h of treatment i.e. 60.05% and 60.60% respectively in comparison to control (Table 6; Figure 6).

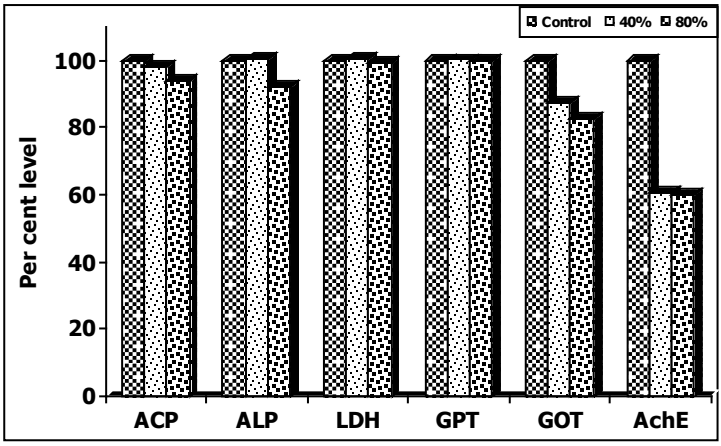

Figure 6: Comparison of acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of malathion at 16 h

4 Discussion

For effective and fast control of termite population synthetic pesticides are used, which show vesry high lethality in different termite species. In the present study fipronil, thiamethoxam and malathion have shown very high lethality, which is proved by very low LD_{50} value. When termites were treated with 40% and 80% of LD_{50} of fipronil, thiamethoxam and malathion each of them

significantly reduced (significant at $p < 0.05$) the level of glycogen i.e. 40.02% and 50% after 16 h of treatment in comparison to control (Table 1-3; Figure 1-3). Similarly, cypermethrin affected the level of glycogen, protein and lipid in *Pimpla turionella* wasp's larvae, pupae and adult females [17]. It also increased the protein level in *Spodoptera litrua* larvae in comparison to control [18]. Similarly 40% and 80% of LD_{50} of malathion, fipronil and thiamethoxam significantly decreased the level of amino acid up to 62.93–87.29% in *O. obesus* after 16 h treatment in comparison to control.

A similar dose of above pesticides caused significant ($p < 0.05$) decrease in lipid, protein and amino acid levels after 4 hour of treatment in comparison to control (Table 1-3; Figure 1-3). These insecticides have also significantly cut down the level of both DNA and RNA levels were found to be decreased after 16 h of treatment. Similarly, amount of total lipids was also increased. It may be due to breakdown of glycerides and diglycerides. However, lipid reserves are built up during active feeding. On the other hand, mobilization of more lipids may induce hydrolysis of triglycerides, diglycerides by an enzyme lipase. Reduction in protein synthesis may lead to decrease in protein concentration which lead to physiological stress in insects.

Similarly, chloropyrifos, thiamethoxam, fipronil, and malathion caused significant depletion in total protein in haemolymph and fat body of silk worm *Bombyx mori* [19]. Malathion caused lipid depletion in haemolymph, fat body and oocytes of *Tenebrio molitor* [20]. It indicates that why more toxic stress in termites and more utilization of lipids occurs. To supplement the nutrients more carbohydrates are converted into lipids during development period in insects [21]. Similarly, glycogen depletion indicates more and more utilization of food reserve for production ATP to cope up the insecticide-induced stress [22, 23]. However, with in the body free glycogen floats in the haemolymph after its breakdown, which induce help to maintain glucose level. This, instant breakdown of glycogen may induce glycogenolysis in insect tissues and rapid utilization of glycogen units in response to stress caused by pesticide treatment [24]. An increase in glycogenesis causes a significant decrease in free amino acid level [25]. Similarly, protein and nucleic acid synthesis may also block at cellular level and catabolism get increase, which results into low availability of proteins and nucleic acid. Hence, the level of amino acid increased after protein catabolism.

In a similar experiment when termites were treated with 40% and 80% of LD_{50} of malathion, fipronil and thiamethaxm showed a significant ($p < 0.05$) increase in acid phosphoatase (113.60 to 101.70), alkaline phosphatase (124.64 to 100.63), glutamate pyruvate transaminase (107.68 to 100.20) and glutamate

oxaloacetate transaminase (110.83 to 101.31) and lactic dehydrogenase (105.78 to 100.21) level after 4 h of treatment in comparison to control (Table 4-6; Figure 4-6). Contrary to this a significant decrease (60.60–6.12%) was observed in acetyl cholinesterase level after 16 h of treatment in comparison to control (Table 4-6; Figure 4-6).

In addition to it all three pesticides significantly ($p < 0.05$) cut down the level of acetylcholinesterase upto 60–61% after 4 hr that proves nuerotoxic effects of these pesticides in termites. Similarly, phenolic compounds such as phosphorus oxycholride showed acetylcholinesterase inhibition at sub lethal dose in subterranean termite C. formosanus [26]. Phosphorus oxycholride induce brain acetylcholinesterase in houseflies and selectively bind to acetylcholinesterase in comparison to other serine hydrolases. Contrary to this malathion also potentially inhibits acetylcholinesterase activity more than malaxon and isomalathion [27]. Similarly, dimethyl maleate inhibits acetylcholinesterase activity at concentration greater than 10 mM [28]. Besides this, malathion [S-(1, 2-dicarboethoxyethyl) O, O-dimethyl phosphorodithiote] shows genotoxicity in treated insects [29]. To fight against toxic insects show significant induction in hydrolytic activities with in the body tissues, which cut down the acid and alkaline phosphatase level [30, 31]. Moreover, few natural pesticides such as pyrethroids inhibit the phosphatase activity in insects [32]. Similarly, in presence of pesticides, transamination of amino acids is increased, which affect the level of glutamate pyruvate transaminase and glutamate oxalo acetate transaminase enzymes [33]. Similarly, increase in lactic dehydrogenase level induce tissue necrosis in insects while increase in alkaline phosphatase level induce lysosomal activities in cells, which leads to biochemical stress in insects [34]. Therefore, a decline in the level of above enzymes directly effect oxygen consumption in insects. More specifically, both fat body and heamolymph exhibit higher glutamate oxaloacetate transaminae activity than the glutamate pyruvate transaminase during successive developmental stages. However, the heamolymph aminotransferase is found significantly decreased [33]. In the present study elevation or reduction in enzyme level is associated with metabolic alterations in insects [35]. which lead to the death of insects after insecticide poisoning [36]. Present study proves termiticidal effects of malathion, fipronil and thiamethaxm on *Odontotermes obesus* which significantly altered the level of important metabolites and enzymes.

Acknowledgements

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Termiticidal effects of *Capparis decidua* on biochemical and enzymatic parameters of *Odontotermes obesus* (Isoptera: Termitidae)

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Abstract. In the present investigation termiticidal effects of *Capparis decidua* and its combinatorial mixtures on biochemical and enzymatic parameters of *Odontotermes obesus* (Isoptera: Termitidae) were observed. *C. decidua* has shown very high termiticidal activity to *O. obesus* (Rambur) when termites were exposed with 40% and 80% of LD_{50}. It's aqueous extract and combinatorial mixtures significantly ($p < 0.05$) inhibited the level of glycogen, amino acid, lipid, DNA, RNA and protein in termites. Besides this, both single and combinatorial mixtures of *C. decidua* significantly ($p < 0.05$) decreased the level of acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase enzymes at very low concentrations after 8 h treatment. More specifically, aqueous extract and combinatorial mixtures significantly inhibited the AChE activity, which the confirms the presence of neurotoxic compounds. However, it can be concluded that *C. decidua* active ingredients can easily kill field termites if used in the poison baits and as fumigant in storehouses.

Keywords: *Odontotermes obesus*, biorganic termiticides, lipid, protein, glycogen, enzymes

1 Introduction

Termites are highly destructive polyphagous insect pests, which damage cereal crops, forest products and are serious problems to the farmers, tree growers and builders throughout the world. In the past, synthetic pesticides were massively used for the fast and effective control of termite population but that has led to pesticide resistance. But, such pesticides are highly toxic to non-target organisms and contaminate the environment. Hence, their alternatives are under exploration to have more potent herbal pesticides. There is a great need of novel pesticidal compositions containing no synthetic pesticide to be used against termites. In addition, there is a need for effective termiticide, which can suppress the regenerative activity in termites at a very mild dose. More specifically, plant species having strong anti-termiteic properties have been searched [1]. In this regard, few plants such as *Polygonal hydro piper* and *Progesterone parviflorus* caused significant mortality and repellent activity in termite, *R. herperus* and *O. assamensis*. Similar termiticidal activity are reported in aqueous and solvent extracts of *Geranium*, *Morus*, *Artemisia*, *Diospyros*, *Crataegus*, *Curcuma*, *Rubia*, *Polygonal*, *Gardenia*, *Cornus*, *Uncaria*, *Rheum*, *Terminalia* and *Saussurea* [2]. Besides this, monoterpenoids isolated from *Flourensia cernua* effectively control termites [3]. Moreover, Alaska yellow and red cedar (*Chamaecyparis nootkatensis*) and redwood (*Sequoia sempervirens*) have shown high antifeedant and toxic activities against termites [4].

Few other plant derivatives such as 2-methyl-anthraquinone, plumbagin, diosindgo, diospyrin, isodiospyrin and microphyllone isolated from *Diospyros sylvatica* root [5]. and Cedrol [6] isolated from *Juniperus procera* exhibited very high mortality in *O. obesus*. Similarly 2, 2': 5', 2"-Terthiophene and 5'-(3-buten-1-ynyl)-2,2'-bithiophene showed 100% mortality in *C. formosanus* [7]. Similarly, flavanoids, geninstein, biochantin A, apigenin, querceptin and glyceollin reduce fecundity and food consumption Formosan subterranean termites [8]. Moreover, Sesquiterpenes, (4S)-2, 6, 10-bisaboratrien-4-ol-1-one, 1, 8-epoxy-1(6), 2, 4, 10-bisaborpenta-en-4-ol (2), and 1-methoxy-4-cadinene (3) isolated from the black heartwood of *Cryptomeria japonica* [9, 10].

Capparis decidua is a native plant commonly known as 'Kureel' in Hindi, belongs to family Capparidaceae. It is a densely branched shrub with scanty, small, caduceus leaves. Barks, leaves and roots of *C. decidua* have been claimed to relieve variety of ailments such as toothache, cough and asthma. In the present study, termiticidal effects of *C. decidua* and its mixtures were observed on biochemical and enzymatic parameters of Odontotermes obesus.

2 Material and methods

Collection of termites and plant material

Termite, *Odontotermes obesus* (Rambur) workers were collected from the University garden and temporary culture was maintained in the laboratory at 37°C ± 2°C at 80% RH by providing green leaves as food material. Termite culture was protected from light illumination, by using black paper sheets wrapped around the glass containers (12 × 9 inch). Capparis decidua collected from semi arid regions of Rajasthan. Standard taxonomic key was applied for the proper identification of plant.

Preparation of extracts

C. decidua stem were chopped in small pieces and milled to make powder, weighed and solubilized in the water. The solibilized extracts were filtered with Whatmann paper. No 1 and were concentrated under vacuum (30°C). After evaporation of water, it was weighed and solubilized in known volume of distilled water.

Capparis decidua and other ingredients used in preparation of combinatorial mixtures

	Combinatorial ingredients added mixtures
C-ST	*Capparis decidua* stem powder (180 gm) + Coconut oil (50ml) + Terpene oil (50ml) + Glycerol (50 ml) + Sulphur (11) + Water (15 liter)
C-BT	*Capparis decidua* stem powder (180 gm) + Coconut oil (50ml) + Terpene oil (50ml) + Glycerol (50 ml) + Borate (11) + Water (15 liter)
C-CoT	*Capparis decidua* stem powder (180 gm) + Coconut oil (50ml) + Terpene oil (50ml) + Glycerol (50 ml) + Copper (11) + Water (15 liter)
PCU	Photoactivated cow urine (48 h) (10 μg/μl)
C-CuT	*Capparis decidua* stem powder (180 gm) + Photoactivated Cow urine (15 liter)
Capparis	*Capparis decidua* stem powder 6gm/l of water aqueous extract

Toxicity determination

Toxicity bioassays were conducted in the laboratory and LD_{50} of each mixture was determined separately. Toxicity experiments were conducted by using increasing concentration of each mixture i.e. 24 μg, 48 μg, 96 μg, 192 μg, 384 μg, 768 μg, and 1536μg. The treatment mixtures were then coated on cellulose paper (size 1X1 cm2), air dried and kept in the central area of Petri dish. Treatment and controls were tested in triplicate for each mixture. In each Petri dishes 125 termites (1 gm weight) were released and termite mortality and survival were observed at different periods. Dead termites were separated from the alive on the basis of body movement. LD_{50} values were determined by Probit method [11]. LD_{50} values were calculated in μg/gm body weight of termites 16 h after treatment.

Determination of biomolecular parameters

Termite workers were treated with 40% and 80% of LD_{50} (22.68 μg/gm, 13.00 μg/gm, 1.56 μg/gm, 19.20 μg/gm, 15.68 μg/gm and 26 μg/gm body weight of termite for C-ST, C-BT, C-CoT, PCU, C-CuT and aquous extract of *C. decidua respectively*). Termites were sacrificed, homogenized and centrifuged to prepare their whole body extracts for biomolecular estimation. Changes in the level of various biomolecules were measured after various time intervals i.e. 4, 8, 12 and 16h. Few important biomolecules such as glycogen, total free amino acids, total lipid, nucleic acids (DNA and RNA) and total protein were determined.

Determination of glycogen

Glycogen contents were measured according to method of Dubois et al. [12]. For this purpose 500 mg of termites were homogenized in 2ml of 5% Trichloro acetic acid with the help of glass-glass homogenizer and centrifuged. Optical density of the reactant was read at 530nm. Glycogen contents in unknown (supernatant) were calculated by using standard curve drawn with known amount of glucose. The blank was set by taking 0.50ml of 5% TCA and 6 ml of concentrate H2SO4. The amount of glycogen was expressed in gm/100gm of body weight of termites. Three treatments were performed at three trials. Data obtained was statistically analyzed by using ANOVA method.

Determination of total free amino acid

Level of free amino acids was determined following Spies et al. [13]. A total 500 mg of termites were homogeninzed in 2 ml of 95% ethyl alcohol. Homogenate was centrifuged at $15,000\times$ g for 20 minutes and supernatant was separated. For estimation of total free amino acids 0.1 ml of supernatant was taken and to it 0.1 ml of distilled water and 2.0 ml Ninhydrin reagent were mixed. The reaction mixture was kept in boiling water for 15 minutes. A total of 2 ml of 5.0 % ethyl alcohol was added to the above boiled mixture. A violet color was developed in the reaction mixture which was measured at 575 nm. For calculating the total free amino acid content standard curve was prepared by using known amount of glycine and was expressed in gm/100 gm body weight of termites. Three replicates were used and data is statistically analyzed by ANOVA method.

Determination of total Lipid

Level of total lipid in whole body extracts of termite was estimated according to method of Floch et al. [14]. A total of 500 mg of termite workers were homogenized in 5 ml of chloroform and methanol mixture (2:1 v/v). Total lipid contents were weighted at the end and expressed in gm/100 gm body weight of termites. Three replicates were set and data was statistically analyzed by ANOVA method.

Determination of nucleic acids

Level of nucleic acids in the whole body extracts of termites was estimated according to method of Scheidner et al. [14]. For this purpose a total 500 mg of termite workers were fed with 40% and 80% of LD_{50} (22.68 μg/gm, 13.00 μg/gm, 1.56 μg/gm, 19.20 μg/gm, 15.68 μg/gm and 26 μg/gm body weight of termite for C-ST, C-BT, C-CoT, PCU, C-CuT and aquous extract of *C. decidua respectively*) separately. Insects were scarified and homogenized in 5%TCA with glass-glass homogenizer at 15,000 $\times$ g for 25 minutes.

DNA estimation

For DNA estimation, 0.2 ml of supernatant was taken and it was diluted by adding 3.8 ml of distilled water. Then 4.0 ml of diphenylamine reagent (1 gm of diphenylamine, 100 glacial acetic acid and 2.5 ml of conc. H_2SO_4) were added to it. The mixtures were kept in boiling water bath for 10 minutes. A blue color was developed in the solution which is measured at 595 nm (O.D.).

RNA estimation

For RNA estimation 0.2 ml of supernatant was taken and it was diluted by adding 4.8ml of distilled water. Now 2ml of orcinol reagent (1 gm orcinol, 100 ml conc. HCl and 0.5 gm ferric acid) was added to it. The solution was kept in boiling water bath for 10 minutes, a green color was developed, which was measured at 660nm. In both cases three replicates were set and data obtained was stastically analyzed by ANOVA method.

Determination of total protein

Total proteins of termites were estimated according to Lowry et al. [16]. For this purpose 500mg of termite workers were treated with 40% and 80% of LD_{50} (22.68 μg/gm, 13.00 μg/gm, 1.56 μg/gm, 19.20 μg/gm, 15.68 μg/gm and 26 μg/gm body weight of termite for C-ST, C-BT, C-CoT, PCU, C-CuT and aquous extract of *C. decidua* respectively). These treated termites were homogenized in 4.0 ml of 10% TCA with the help of glass-glass homogenizer. The obtained homogenate was centrifuged at 15,000 $\times$ g for 15 minutes. Each experiment was performed three times. Standard curve was prepared by using 10 μg, 20 μg, 40 μg, 80 μg and 100μg of Bovine serum albumen. Data obtained was statistically analyzed by ANOVA method.

In vivo Determination of enzymatic parameters

To observe the effect on enzymatic parameters 500 mg of adult termite workers were provided sub-lethal doses (40% and 80% of LD_{50}) of C-ST, C-BT, C-CoT, PCU, C-CuT and aquous extract of *C. decidua* with the cellulose paper as diet. Insects were sacrificed at the 4 h interval up to 16 h for measurement of various enzyme levels. Insects were homogenized in phosphate saline buffer (pH 6.9) in a glass-glass homogenizer and centrifuged at 4 °C for 25 minutes at 15,000 $\times$ g. Supernatant was isolated in a glass tube and used as enzyme source.

Determination of acid and alkaline phosphatase

Level of alkaline phophatase level was determined according to the method of Bergmeyer [17]. For this purpose 500 mg of termites were homogenized in 1 ml of PBS buffer at 4 °C and centrifuged at 15,000 $\times$ g for 15 min. A 0.2 ml of supernatant was taken in a test tube and 1.0 ml of acid buffer substrate solution was added. Contents were mixed thoroughly and incubated for 30 minutes at 37 °C. Now 4.0 ml of 0.10N NaOH solution was added to the incubation mixture. Similarly, for determination of ALP, 0.10 ml of supernatant was taken

in a test tube and 1.0 ml of alkaline buffer substrate was mixed with it. The mixture was mixed thoroughly and incubated for 30 minutes at 37 °. Now 5.0 ml of 0.02 N NaOH was added to the incubation mixture. The reaction was stopped by adding excess of NaOH. The p-nitrophenol formed as result of hydrolysis of p-nitrophenyl phosphate gave a yellow colour with NaOH. Optical density was measured at 420 nm. Standard curve was drawn with the help of different concentrations of p-nitrophenol. Enzyme activity was expressed as μ moles of p-nitrophenol formed /30min/mg protein.

Determination of lactic dehydrogenase

Activity of lactic dehydrogease was measured according to the method of Annon [18]. For this purpose, 100 mg of insects were homogenized in 1.0 ml of 0.1 M phosphate buffer (pH 7.5) in ice bath and centrifuged at 10000 × g for 30 minutes in cold centrifuge at 4 °. Supernatant was used as enzyme source. For determination of enzyme activity 0.05 ml of enzyme source was added to 0.50 ml of pyruvate substrate. Now the contents were incubated at 37 °C for 45 minutes. Now 0.50 ml of 2,4- dinitrophenyl hydrazine solution was added and the contents were mixture and kept at the room temperature. After 20 minutes, 5.0 ml of 0.4 N NaOH was mixed and left for 30 minutes at room temperature. The optical density was measured at 540 nm and it was converted to LDH unit by drawing a standard curve. Enzyme activity has been expressed as m moles of pyruvate reduced/45min/mg protein.

Determination of glutamate pyruvate transaminase and glutamic-oxaolacetic transaminase

GPT and GOT activity was measured according to the method of Reitman and Frankel [19]. A total of 500 mg termites were homogenized in 2 ml ice cold PBS buffer and centrifuged at 15,000 × g for 15 min at 4 °C. For determining the activity of GPT, 0.10 ml of enzyme source was taken and 0.50 ml of GPT substarte. Similarly, for determination of GOT, 0.10 ml of enzyme source was taken and 0.50 ml of GOT substrate was added to it. Now 0.50 ml of 2, 4-dinitrophenyl hydrazine solution was added and contents were left stand for 15 minutes at room temperature. Then 5.0 ml of 0.4 N NaOH was added and mixed well and allowed to stand at room temperature for 20 minutes. The optical density was read at 505 nm after setting the blank. Standard curve was prepared by using oxaloacetic acid as working standard. The enzyme activity was expressed in units of glutamate pyruvate transaminase or glutamate oxaloacetate transaminase activity/ hr/mg protein.

Determination of acetylcholinesterase

Acetylcholinesterase activity was determined according to the method of Ellman et al. [20]. For this purpose 500mg treated termites were homogenized 50 mM phosphate buffer (pH 8) in ice bath and centrifuged at 1000 × g for 30 minutes in cold centrifuge at 4 °C. To the supernatant 0.10 ml (5×10^{-4} M) of freshly prepared acetylcholinethioiodide solution, 0.05 ml of DTNB reagent (chromogenic agent) and 1.45 ml of PBS (pH 6.9) were added. The changes in optical density were monitored at 412 nm regularly for three minutes at 25 °C. Enzyme activity has been expressed as m moles 'SH' hydrolysed per minute per mg protein.

Statistical analysis

The LD_{50} for each extract was determined by using Probit analysis. Mean, standard deviation, standard error and Student t-test were applied by ANOVA program [21]

3 Results

Toxicity determination

In the present study both Capparis decidua and its combinatorial mixtures have shown very high termiticidal activity as LD_{50} values obtained were very low i.e. 22.68, 13.00, 1.56, 19.20, 15.68 and 26μg/gm body weight of termites for C-ST, C-BT, C-CoT, PCU, C-CuT and aqueous extract of *C. decidua* respectively.

In vivo treatment of 40% and 80% after 16h LD_{50} of C-ST mixture significantly ($p < 0.05$) cut down the level of glycogen, amino acid, lipid, DNA, RNA and protein i.e. 47.23% and 45.09%; 84.87% and 87.41%; 78.58% and 75.71%; 73.41% and 67.64%; 77.14% and 73.26%; 68.07% and 59.61% respectively (Table 1).

Similarly 40% and 80% of 16h LD_{50} of C-BT mixture caused significant ($p < 0.05$) decrease in glycogen level i.e. 39.26% and 48.46% ($p < 0.05$). Similarly amino acid level was found to be decreased up to 83.94% and 77.94% at similar dose of C-BT mixture (Table 2).

In another experiment 40% and 80% of LD_{50} of C-CoT mixture caused significant decrease in glycogen level i.e. 65.03% and 53.37% after 4 h of treatment. Same mixture also caused increase in amino acid level i.e. 105.89% and 96.30% at 4 h of treatment (Table 3). While a significant ($p < 0.05$)

decrease was noted in lipid level at 16 h of treatment i.e. 56.59% and 66.44% in comparison to control respectively. Besides this, DNA and RNA levels were also found to be decreased i.e. 64.35% and 77.17% and 76.93% and 63.87% after 16 h of treatment in comparison to control. Same mixture caused a significant ($p < 0.05$) decrease in protein level i.e. 52.69% and 62.50% at 4 h respectively (Table 3).

In the fourth set of experiment 40% of LD_{50} of photoactivated cow urine (PCU) caused maximum decrease in glycogen level i.e. 42.33% in comparison to control. Similarly amino acid level was found to be decreased up to 83.49% after 16 h of treatment, while lipid level was found to be decreased i.e. 81.16% and 55.83% in comparison to control respectively. A similar dose of photoactivated cow urine caused decrease in DNA and RNA level i.e. 77.41% and 62.82% and 52.95% and 57.55% after 16 h treatment in comparison to control respectively. Similar total protein level was also found to be decreased up to 52.88% and 50.76% after 16 h of treatment respectively (Table 4).

Similarly C-CuT mixture caused significant decrease in glycogen level 45.09% and 29.57% (Table 5) and amino acid level i.e. 87.41% and 78.98% after 16 h treatment of termite with 40% and 80% LD_{50} of C-CuT mixture. Same mixture also caused regular decrease in lipid levels up to 16 h i.e 75.71% and 71.13% in comparison to control respectively. A similar dose significantly ($p < 0.05$) cut down the level of both DNA and RNA levels i.e. 67.64% and 67.17% and 73.26% and 52.55% respectively (Table 5). C-CuT mixture also caused significant ($p < 0.05$) decrease in protein level up to 16 h i.e. 59.61% and 46.73% in comparison to control respectively (Table 5).

40% of *C. decidua* aqueous extract caused significant (p$>$ 0.05) decreases in glycogen level in treated termites in comparison to control at 16 h of treatment. Maximum decrease in glycogen level i.e. 67.17% was observed after 16 h of treatment of 80% of LD_{50} of *C. decidua* aqueous extract. A similar dose of *C. decidua* aqueous extract caused very slight decrease in lipid contents after 4 h of treatment. Later on it was found to be significantly (p$>$ 0.05) decreased in other successive treatments. Similarly DNA level was also significantly decreased after 16 h of treatment i.e. 77.53% and 73.88% in comparison to control. Besides this, both RNA and protein levels were found to be decreased when termites were treated with 40% and 80% of LD_{50} of *C. decidua* aqueous extract i.e. 94.08% and 90.92% and 66.73% and 64.54% after 16 h treatment in comparison to control respectively (Table 6).

Table 1: Effect of 40% and 80% of LD_{50} of *C*-ST mixture on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.0115 (100)	1.99*±0.043 (61.04)	1.65*±0.021 (50.61)	1.81*±0.034 (55.52)	1.93*±0.028 (59.20)	1.73*±0.027 (53.06)	1.66*±0.045 (50.92)	1.54*±0.02 (47.23)	1.47*±0.013 (45.09)
Amino acid	0.866±0.003 (100)	0.868*±0.0058 (100.23)	0.835*±0.0015 (96.42)	0.859*±0.0048 (99.19)	0.783*±0.013 (90.41)	0.75*±0.0023 (86.60)	0.793*±0.001 (91.57)	0.735*±0.005 (84.87)	0.757*±0.002 (87.41)
Lipid	1.046±0.001 (100)	0.982*±0.0029 (93.88)	0.97*±0.014 (92.73)	0.985*±0.0035 (94.16)	0.95*±0.003 (90.82)	.917*±0.003 (87.66)	0.92*±0.0018 (87.95)	0.822*±0.0058 (78.58)	0.792*±0.0088 (75.71)
D.N.A.	0.85±0.028 (100)	0.744*±0.003 (87.52)	0.727*±0.002 (85.52)	0.707*±0.0038 (83.17)	0.714*±0.0027 (84.00)	0.713*±0.002 (83.88)	0.642*±0.003 (75.52)	0.624*±0.0037 (73.41)	0.575*±0.004 (67.64)
R.N.A.	0.98±0.023 (100)	0.925*±0.0093 (94.38)	0.884*±0.0013 (90.20)	0.872*±0.0025 (88.97)	0.854*±0.007 (87.14)	0.818*±0.005 (83.46)	0.845*±0.002 (86.22)	0.756*±0.0037 (77.14)	0.718*±0.0015 (73.26)
Protein	5.2±0.046 (100)	3.5*±0.034 (67.30)	3.52*±0.006 (67.69)	3.43*±0.012 (65.96)	3.56*±0.012 (68.46)	3.63*±0.014 (69.80)	3.38*±0.021 (65.00)	3.54*±0.023 (68.07)	3.10*±0.029 (59.61)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

Table 2: Effect of 40% and 80% of LD_{50} of *C*-BT on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.0115 (100)	1.61*±0.045 (49.38)	2.05*±0.029 (62.88)	1.89*±0.038 (57.97)	2.27*±0.037 (69.63)	1.31*±0.04 (40.18)	1.78*±0.029 (54.60)	1.28*±0.07 (39.26)	1.58*±0.034 (48.46)
Amino acid	0.866±0.003 (100)	0.876*±0.028 (107.15)	1.15*±0.011 (132.79)	0.769*±0.0038 (88.79)	0.763*±0.011 (88.10)	0.783*±0.003 (90.41)	0.763*±0.003 (88.10)	0.727*±0.0067 (83.94)	0.675*±0.006 (77.94)
Lipid	1.046±0.001 (100)	0.983*±0.004 (93.97)	1.27*±0.027 (121.41)	0.953*±0.0045 (91.10)	0.854*±0.0065 (81.64)	1.07*±0.028 (99.63)	0.934*±0.0038 (89.29)	0.909*±0.0017 (86.90)	0.823*±0.002 (78.68)
D.N.A.	0.85±0.028 (100)	0.722*±0.0038 (84.94)	0.649*±0.0026 (76.35)	0.714*±0.0028 (84.00)	0.639*±0.003 (75.17)	0.55*±0.0034 (64.70)	0.585*±0.0045 (68.82)	0.713*±0.0038 (83.88)	0.539*±0.009 (63.41)
R.N.A.	0.98±0.023 (100)	0.879*±0.0065 (89.69)	0.921*±0.0032 (93.97)	0.848*±0.0045 (86.53)	0.909*±0.0035 (92.75)	0.732*±0.003 (74.69)	0.896*±0.0014 (91.42)	0.633*±0.005 (64.59)	0.884*±0.005 (90.20)
Protein	5.2±0.046 (100)	2.92*±0.03 (56.15)	3.08*±0.04 (59.23)	2.93*±0.0045 (56.34)	2.56*±0.012 (49.23)	2.38*±0.034 (45.76)	2.08*±0.043 (40.00)	2.34*±0.067 (45.00)	2.54*±0.028 (48.84)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

Table 3: Effect of 40% and 80% of LD_{50} of *C*-CoT on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.0115 (100)	2.12*±0.014 (65.03)	1.74*±0.023 (53.37)	2.04*±0.018 (62.57)	1.86*±0.009 (57.05)	1.93*±0.005 (59.20)	1.62*±0.0042 (49.69)	1.67*±0.041 (51.23)	1.54*±0.023 (47.23)
Amino acid	0.866±0.003 (100)	0.917*±0.003 (105.89)	0.834*±0.0017 (96.30)	0.578*±0.012 (66.74)	0.792*±0.009 (91.45)	0.545*±0.002 (62.93)	0.766*±0.0012 (88.45)	0.527*±0.002 (60.85)	0.674*±0.0023 (77.82)
Lipid	1.046±0.001 (100)	0.975*±0.009 (93.21)	0.925*±0.002 (88.43)	0.892*±0.0025 (85.27)	0.756*±0.0012 (72.27)	0.713*±0.003 (68.16)	0.822*±0.0012 (78.58)	0.592*±0.0028 (56.59)	0.695*±0.0088 (66.44)
D.N.A.	0.85±0.028 (100)	0.723*±0.009 (85.06)	0.742*±0.0012 (87.29)	0.69*±0.004 (81.17)	0.715*±0.0014 (84.12)	0.672*±0.001 (79.06)	0.673*±0.0057 (79.17)	0.547*±0.0057 (64.35)	0.656*±0.0012 (77.17)
R.N.A.	0.98±0.023 (100)	0.913*±0.0012 (93.16)	0.885*±0.0022 (90.30)	0.876*±0.0059 (89.38)	0.865*±0.0021 (88.26)	0.79*±0.0042 (80.61)	0.715*±0.0018 (72.95)	0.754*±0.0017 (76.93)	0.626*±0.0025 (63.87)
Protein	5.2±0.046 (100)	2.74*±0.017 (52.69)	3.25*±0.051 (62.50)	2.82*±0.009 (54.23)	3.15*±0.024 (60.57)	3.11*±0.018 (59.80)	3.45*±0.023 (66.34)	3.23*±0.022 (62.12)	3.27*±0.012 (62.88)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

Table 4: Effect of 40% and 80% of LD_{50} of photoactivated cow urine on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.0115 (100)	2.22*±0.023 (68.09)	2.22*±0.012 (68.09)	1.94*±0.037 (59.50)	1.86*±0.015 (57.05)	1.62*±0.018 (49.69)	1.63*±0.002 (50.00)	1.38*±0.013 (42.33)	1.52*±0.0057 (46.62)
Amino acid	0.866±0.003 (100)	0.871*±0.0036 (100.57)	0.725*±0.0012 (83.71)	0.845*±0.0038 (97.57)	0.829*±0.0032 (95.72)	0.784*±0.004 (90.53)	0.756*±0.0015 (87.29)	0.774*±0.0032 (89.37)	0.723*±0.0025 (83.49)
Lipid	1.046±0.001 (100)	0.864*±0.02 (82.60)	0.745*±0.002 (71.22)	0.834*±0.003 (79.73)	0.707*±0.004 (67.59)	0.888*±0.005 (84.89)	0.694*±0.002 (66.34)	0.849*±0.004 (81.16)	0.584*±0.0015 (55.83)
D.N.A.	0.85±0.028 (100)	0.733*±0.004 (86.23)	0.654*±0.0017 (76.94)	0.662*±0.01 (77.88)	0.683*±0.0012 (80.35)	0.642*±0.014 (75.52)	0.655*±0.002 (77.05)	0.658*±0.007 (77.41)	0.534*±0.0023 (62.82)
R.N.A.	0.98±0.023 (100)	0.719*±0.0046 (73.36)	0.735*±0.0023 (75.00)	0.755*±0.0037 (77.04)	0.696*±0.0012 (71.02)	0.635*±0.003 (64.79)	0.629*±0.0012 (64.18)	0.519*±0.0046 (52.95)	0.564*±0.002 (57.55)
Protein	5.2±0.046 (100)	4.14*±0.012 (79.61)	3.34*±0.009 (64.23)	3.83*±0.036 (73.65)	2.06*±0.039 (39.61)	3.46*±0.037 (66.53)	3.35*±0.021 (64.42)	2.75*±0.015 (52.88)	2.64*±0.023 (50.76)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

Table 5: Effect of 40% and 80% of LD_{50} of *C*-CuT on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.0115 (100)	1.65*±0.021 (50.61)	1.53*±0.0012 (46.93)	1.93*±0.028 (59.20)	1.27*±0.0037 (38.95)	1.66*±0.045 (50.92)	1.46*±0.009 (44.78)	1.47*±0.013 (45.09)	0.964*±0.0031 (29.57)
Amino acid	0.866±0.003 (100)	0.835*±0.0015 (96.42)	0.768*±0.0029 (88.68)	0.783*±0.013 (90.41)	0.756*±0.009 (87.29)	0.793*±0.001 (91.57)	0.718*±0.002 (82.91)	0.757*±0.002 (87.41)	0.684±0.0012 (78.98)
Lipid	1.046±0.001 (100)	0.97*±0.014 (92.73)	0.921*±0.002 (88.05)	0.95*±0.003 (90.82)	0.925*±0.0011 (88.43)	0.92*±0.0018 (87.95)	0.815*±0.0023 (77.91)	0.792*±0.0088 (75.71)	0.744*±0.0015 (71.13)
D.N.A.	0.85±0.028 (100)	0.727*±0.002 (85.52)	0.67*±0.008 (78.82)	0.714*±0.0027 (84.00)	0.642*±0.01 (75.53)	0.642*±0.003 (75.52)	0.623*±0.0035 (73.29)	0.575*±0.004 (67.64)	0.571*±0.015 (67.17)
R.N.A.	0.98±0.023 (100)	0.884*±0.0013 (90.20)	0.785*±0.0021 (80.10)	0.854*±0.007 (87.14)	0.646*±0.0022 (65.91)	0.845*±0.002 (86.22)	0.635*±0.0012 (64.79)	0.718*±0.0015 (73.26)	0.515*±0.0019 (52.55)
Protein	5.2±0.046 (100)	3.52*±0.006 (67.69)	3.54*±0.017 (68.07)	3.56*±0.012 (68.46)	2.95*±0.017 (56.73)	3.38*±0.021 (65.00)	2.34*±0.017 (45.00)	3.10*±0.029 (59.61)	2.43*±0.026 (46.73)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

Table 6: Effect of 40% and 80% of LD_{50} of *C. decidua* on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.0115 (100)	2.846*±0.0348 (87.30)	2.83*±0.047 (86.81)	2.736*±0.0284 (83.92)	2.62*±0.049 (80.37)	2.526*±0.0272 (77.48)	2.42*±0.049 (74.23)	2.376*±0.0233 (72.88)	2.19*±0.05 (67.17)
Amino acid	0.866±0.003 (100)	0.887*±0.0045 (102.42)	0.886*±0.0061 (102.31)	0.834*±0.0071 (96.30)	0.837*±0.0076 (96.65)	0.812*±0.0026 (93.76)	0.807*±0.0027 (93.19)	0.79*±0.0027 (91.22)	0.777*±0.0042 (89.72)
Lipid	1.046±0.001 (100)	1.028*±0.0034 (98.28)	1.01*±0.0046 (96.56)	0.991*±0.0041 (94.56)	0.971*±0.0084 (92.83)	0.972*±0.0066 (92.75)	0.966*±0.0077 (92.35)	0.974*±0.0065 (92.94)	0.955*±0.0073 (91.30)
D.N.A.	0.85±0.028 (100)	0.836*±0.0056 (98.35)	0.822*±0.0053 (96.71)	0.752*±0.011 (88.47)	0.753*±0.0064 (88.58)	0.685*±0.0038 (80.58)	0.641*±0.0104 (75.41)	0.659*±0.0042 (77.53)	0.628*±0.006 (73.88)
R.N.A.	0.98±0.023 (100)	0.945*±0.0055 (96.43)	0.928*±0.0066 (94.69)	0.936*±0.0074 (95.51)	0.926*±0.0063 (94.49)	0.926*±0.0066 (94.49)	0.906*±0.0034 (92.45)	0.922*±0.0047 (94.08)	0.891*±0.004 (90.92)
Protein	5.2±0.046 (100)	4.78*±0.071 (91.92)	4.296*±0.022 (82.62)	4.25*±0.0284 (81.73)	4.13*±0.029 (79.42)	3.83*±0.023 (73.65)	3.66*±0.037 (70.38)	3.47*±0.061 (66.73)	3.356*±0.075 (64.54)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

In vivo Determination of enzymatic parameters

In a similar experiment when termites were treated with 40% and 80% of LD_{50} of C-ST mixture, it caused a significant ($p > 0.05$) increase in acid phosphatase (107.49% and 107.10%), alkaline phosphatase (105.06% and 104.40%), lactic dehydrogenase (102.65% and 103.29%) and glutamate pyruvate transaminase (106.51% and 106.83%) levels after 4 hr treatment in comparison to control respectively (Table 7). While glutamate oxaloacetate transaminase and acetyl cholinesterase level was found to be decreased after 16 h treatment i.e. 80.03% and 79.73% and 61.12% and 61.09% in comparison to control respectively (Table 7).

Similarly 40% and 80% of LD_{50} of C-BT mixture caused a significant ($p < 0.05$) decrease in alkaline phosphatase (90.08% and 86.21%), glutamate oxaloacetate transaminase (91.26% and 92.19%) and acetyl cholinesterase (78.85% and 79.62%) levels, while slight increase was observed in the acid phosphatase (103.29% and101.61%), lactic dehydrogenase (101.89% and 101.64%) and glutamate pyruvate transaminase (102.46% and 106.01%) levels at 12 h of treatment in comparison to control respectively (Table 8).

However, 40% of LD_{50} of C-CoT mixture caused a significant ($p < 0.05$) increase in the acid phosphatase (104.38%), alkaline phosphatase (113.54%), lactic dehydrogenase (101.53%), glutamate pyruvate transaminase (106.11%) and glutamate oxaloacetate transaminase levels (101.31%), while 80% of LD_{50} of C-CoT mixture caused significant decrease in alkaline phosphatase (89.54%), lactic dehydrogenase (99.62%), glutamate pyruvate transaminase (99.41%), glutamate oxaloacetate transaminase (86.97%) and acetyl cholinesterase (74.02%) levels after 12 h of treatment of termites (Table 9).

Similarly 40% of LD_{50} of photoactivated cow urine caused a significant ($p < 0.05$) decrease in the acid phosphatase (98.52%), lactic dehydrogenase (99.68%), glutamate pyruvate transaminase (70.44%) and glutamate oxaloacetate transaminase (99.74%) levels after 4 h of treatment in comparison to control respectively. While 80% of LD_{50} of photoactivated cow urine caused a significant ($p < 0.05$) increase in acid phosphoatase (107.10%), alkaline phosphatase (105.60%), lactic dehydrogenase (103.24%) and glutamate pyruvate transaminase levels (106.51%), while decrease in the glutamate oxaloacetate transaminase (98.78%) and acetyl cholinesterase (97.98%) level after 4 h of treatment in comparison to control respectively (Table 10).

Table 7: Effect of 40% and 80% of LD_{50} of *C*-ST on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85±0.022 (100.00)	5.21*±0.0088 (107.49)	5.19*±0.0078 (107.10)	5.11*±0.015 (105.36)	5.06*±0.088 (104.46)	4.93*±0.0328 (101.71)	4.91*±0.0058 (101.23)	4.50*±0.0088 (92.71)	4.62*±0.013 (95.32)
ALP	1.25±0.0057 (100.00)	1.31*±0.015 (105.06)	1.31*±0.0058 (104.40)	1.29*±0.002 (103.22)	1.30*±0.00031 (103.80)	1.28*±0.0017 (102.48)	1.11*±0.0052 (89.54)	1.13*±0.016 (90.40)	1.06*±0.0088 (85.33)
LDH	14.59±0.039 (100.00)	14.97*±0.02 (102.65)	15.07*±0.0058 (103.29)	14.89*±0.013 (102.10)	14.93*±0.0087 (102.29)	14.87*±0.0058 (101.91)	14.53*±0.022 (99.62)	14.57*±0.02 (99.90)	14.23*±0.012 (97.57)
GPT	6.55±0.015 (100.00)	6.97*±0.0033 (106.51)	6.99*±0.0091 (106.83)	6.88*±0.012 (105.13)	6.72*±0.0039 (102.55)	6.73*±0.018 (102.79)	6.51*±0.0012 (99.41)	6.46*±0.0088 (98.67)	6.41*±0.0024 (97.91)
GOT	1.64±0.023 (100.00)	1.63*±0.0058 (99.39)	1.61*±0.0012 (97.90)	1.56*±0.012 (95.32)	1.57*±0.0062 (95.83)	1.42*±0.012 (86.99)	1.43*±0.0088 (86.97)	1.311*±0.002 (80.03)	1.31*±0.0015 (79.73)
AchE	0.0116±0.009 (100.00)	$0.014^{*}\pm28\cdot10^{-4}$ (123.56)	$0.012^{*}\pm47\cdot10^{-4}$ (104.31)	$0.00893^{*}\pm12\cdot10^{-4}$ (77.01)	$0.0096^{*}\pm2\cdot10^{-4}$ (82.55)	$0.0081^{*}\pm3.4\cdot10^{-4}$ (69.45)	$0.0086^{*}\pm12\cdot10^{-4}$ (74.02)	$0.0071^{*}\pm4.5\cdot10^{-4}$ (61.12)	$0.0071^{*}\pm4.4\cdot10^{-4}$ (61.09)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant at ($P < 0.05$, student t-test)

- acid phosphatase (ACP) and alkaline phosphatase (ALP)- µ moles of p-nitrophenol formed /30 minute/mg protein.
- lactic dehydrogenase (LDH): µ moles of pyruvate reduced/ 30min/mg/protein.
- glutamate-Pyruvate transaminase (GPT): Units of glutamate-pyurvate transaminase activity/hour/mg protein.
- glutamate oxalo acetate transaminase (GOT): Units of glutamate oxalo acetate transaminase activity/ hour/ mg protein.
- Acetylcholine esterase (AchE): µ moles 'SH' hydrolysed/min/mg/protein.

Table 8: Effect of 40% and 80% of LD_{50} of *C*-BT on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85±0.022 (100.00)	5.36*±0.029 (110.65)	5.39*±0.061 (111.07)	5.23*±0.014 (107.97)	5.22*±0.0016 (107.53)	5.01*±0.023 (103.29)	4.93*±0.012 (101.61)	4.63*±0.0176 (95.60)	4.50*±0.036 (92.78)
ALP	1.25±0.0057 (100.00)	1.41*±0.0039 (112.77)	1.41*±0.003 (112.51)	1.25*±0.0012 (100.45)	1.35*±0.0042 (107.81)	1.13*±0.018 (90.08)	1.08*±0.016 (86.21)	0.96*±0.0117 (76.98)	0.893*±0.0061 (71.49)
LDH	14.59±0.039 (100.00)	15.24*±0.02 (104.47)	15.15*±0.021 (103.83)	15.11*±0.014 (103.58)	15.05*±0.02 (103.12)	14.86*±0.0088 (101.89)	14.83*±0.0058 (101.64)	14.54*±0.014 (99.68)	14.37*±0.0136 (98.48)
GPT	6.55±0.015 (100.00)	7.14*±0.012 (109.06)	7.24*±0.023 (110.58)	6.82*±0.0088 (104.17)	7.05*±0.021 (107.67)	6.71*±0.010 (102.46)	6.94*±0.021 (106.01)	6.46*±0.0090 (98.66)	6.52*±0.0087 (99.60)
GOT	1.64±0.023 (100.00)	1.62*±0.0024 (99.01)	1.61*±0.0058 (98.11)	1.59*±0.0041 (96.99)	1.57*±0.0038 (96.02)	1.50*±0.0088 (91.26)	1.51*±0.00115 (92.19)	1.41*±0.0145 (86.13)	1.40*±0.002 (85.65)
AchE	0.0116±0.009 (100.00)	$0.0116*\pm24\cdot10^{-4}$ (100.57)	$0.0115*\pm12\cdot10^{-4}$ (99.71)	$0.0096*\pm1.7\cdot10^{-4}$ (82.84)	$0.0098*\pm2,2\cdot10^{-4}$ (84.79)	$0.0091*\pm1,7\cdot10^{-4}$ (78.85)	$0.0092*\pm1.5\cdot10^{-4}$ (79.62)	0.0082*±0.0032 (70.38)	$0.0083*\pm1.2\cdot10^{-4}$ (71.17)

Table 9: Effect of 40% and 80% of LD_{50} of *C*-CoT on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85±0.022 (100.00)	5.51*±0.0048 (113.55)	5.19*±0.0078 (107.10)	5.46*±0.0051 (112.69)	5.06*±0.0088 (104.46)	5.06*±0.026 (104.38)	4.91*±0.0058 (101.23)	4.94*±0.017 (101.75)	4.72±0.0058 (97.31)
ALP	1.25±0.0057 (100.00)	1.56*±0.012 (124.64)	1.31*±0.0058 (104.40)	1.49*±0.0033 (119.46)	1.30*±0.00031 (103.80)	1.42*±0.0047 (113.54)	1.11*±0.0052 (89.54)	1.34*±0.0085 (106.96)	1.29*±0.0078 (103.57)
LDH	14.59±0.039 (100.00)	15.21*±0.0044 (104.22)	15.07*±0.0058 (103.29)	15.03*±0.012 (103.04)	14.93*±0.0087 (102.29)	14.81*±0.028 (101.53)	14.53*±0.022 (99.62)	14.57*±0.009 (99.91)	14.96*±0.015 (102.53)
GPT	6.55±0.015 (100.00)	7.37*±0.0088 (112.62)	6.99*±0.0091 (106.83)	7.17*±0.012 (109.51)	6.72*±0.0039 (102.55)	6.95*±0.01 (106.11)	6.51*±0.0012 (99.41)	6.84*±0.02 (104.52)	6.66*±0.015 (101.67)
GOT	1.64±0.023 (100.00)	1.78*±0.0017 (108.59)	1.61*±0.0012 (97.90)	1.75*±0.0015 (106.89)	1.57*±0.0062 (95.83)	1.66*±0.012 (101.31)	1.43*±0.0088 (86.97)	1.61*±0.0018 (98.45)	1.48*±0.0026 (90.52)
AchE	0.0116±0.009 (100.00)	$0.0118^{*}\pm3\cdot10^{-4}$ (101.72)	$0.012^{*}\pm47\cdot10^{-4}$ (104.31)	$0.014^{*}\pm15\cdot10^{-4}$ (124.13)	$0.0096^{*}\pm2\cdot10^{-4}$ (82.55)	$0.0096^{*}\pm1.7\cdot10^{-4}$ (83.10)	$0.0086^{*}\pm12\cdot10^{-4}$ (74.02)	$0.0092^{*}\pm0.6\cdot10^{-4}$ (79.45)	$0.0086^{*}\pm1.5\cdot10^{-4}$ (74.45)

Table 10: Effect of 40% and 80% of LD_{50} of photoactivated cow urine on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85±0.022 (100.00)	4.78*±0.0044 (98.52)	5.19*±0.0078 (107.1)	4.59*±0.0094 (94.66)	5.06*±0.0088 (104.46)	4.46*±0.0023 (92.03)	4.91*±0.0058 (101.23)	4.26*±0.007 (87.89)	4.62*±0.013 (95.32)
ALP	1.25±0.0057 (100.00)	1.26*±0.0012 (100.51)	1.32*±0.0058 (105.60)	1.246*±0.0024 (99.65)	1.314*±0.0026 (105.14)	1.23*±0.0059 (98.09)	1.17*±0.0115 (93.60)	1.21*±0.0015 (96.56)	1.08*±0.017 (86.40)
LDH	14.59±0.039 (100.00)	14.54*±0.02 (99.68)	15.06*±0.022 (103.24)	14.52*±0.0038 (99.49)	14.96*±0.0058 (102.53)	14.55*±0.0088 (99.71)	14.56*±0.0115 (99.79)	14.43*±0.023 (98.93)	14.37*±0.012 (98.51)
GPT	6.55±0.015 (100.00)	1.93*±0.02 (70.44)	6.98*±0.02 (106.51)	6.53*±0.011 (99.65)	6.76*±0.012 (103.15)	6.44*±0.022 (98.27)	6.66*±0.0012 (101.62)	6.34*±0.0071 (96.76)	6.45*±0.02 (98.42)
GOT	1.64±0.023 (100.00)	1.64*±0.0027 (99.74)	1.62*±0.01 (98.78)	1.62*±0.0015 (98.96)	1.58*±0.0042 (96.21)	1.6*±0.002 (97.83)	1.46*±0.0029 (80.73)	1.58*±0.003 (96.58)	1.33*±0.0065 (80.91)
AchE	0.0116±0.009 (100.00)	$0.0143*\pm28\cdot10^{-4}$ (123.56)	$0.0114\pm79\cdot10^{-4}$ (97.98)	$0.0089*\pm12\cdot10^{-4}$ (77.01)	$0.0097*\pm2\cdot10^{-4}$ (83.64)	$0.0081*\pm3.4\cdot10^{-4}$ (69.45)	$0.0082*\pm3.2\cdot10^{-4}$ (70.26)	$0.0071*\pm4.5\cdot10^{-4}$ (61.12)	$0.007*\pm5.5\cdot10^{-4}$ (60.60)

Table 11: Effect of 40% and 80% of LD_{50} of *C*-CuT on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, Glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85±0.022 (100.00)	4.42*±0.0026 (91.20)	4.39*±0.0023 (90.41)	4.28*±0.013 (88.15)	4.16*±0.00088 (85.72)	4.18*±0.0018 (86.24)	4.04*±0.025 (83.40)	3.95*±0.004 (81.38)	3.81*±0.022 (78.52)
ALP	1.25±0.0057 (100.00)	1.25*±0.00069 (99.83)	1.24*±0.00074 (98.99)	1.23*±0.0017 (98.35)	1.16*±0.0012 (98.99)	1.15*±0.031 (91.76)	1.08*±0.0074 (86.24)	1.06*±0.0031 (84.48)	0.98*±0.00088 (78.59)
LDH	14.59±0.039 (100.00)	14.15*±0.018 (97.01)	13.98*±0.0022 (95.83)	14.15*±0.065 (96.98)	13.65*±0.0029 (93.58)	13.95*±0.0043 (95.60)	13.10*±0.0013 (89.76)	13.15*±0.0005 (90.12)	12.26*±0.023 (84.03)
GPT	6.55±0.015 (100.00)	6.46*±0.025 (98.63)	6.36*±0.013 (97.12)	6.28*±0.0029 (95.95)	6.07*±0.017 (92.74)	6.08*±0.0033 (92.77)	5.57*±0.011 (85.11)	5.68*±0.0036 (86.68)	5.28*±0.0088 (80.56)
GOT	1.64±0.023 (100.00)	1.62*±0.0014 (98.50)	1.61*±0.0026 (98.08)	1.61*±0.0018 (97.85)	1.59*±0.011 (97.14)	1.56*±0.0017 (95.41)	1.29*±0.0015 (78.43)	1.33*±0.0025 (80.85)	1.25*±0.016 (76.44)
AchE	0.0116±0.009 (100.00)	$0.011*\pm58\cdot10^{-4}$ (94.54)	$0.00985*\pm1.2\cdot10^{-4}$ (84.89)	$0.0096*\pm1.7\cdot10^{-4}$ (83.10)	$0.0092*\pm0.1\cdot10^{-4}$ (79.05)	$0.00923*\pm1.5\cdot10^{-4}$ (79.60)	$0.0089*\pm5.5\cdot10^{-4}$ (76.32)	$0.008\pm2.6\cdot10^{-4}$ (76.72)	$0.0082*\pm43\cdot10^{-4}$ (70.72)

Table 12: Effect of 40% and 80% of LD_{50} of *Capparis decidua* aqueous extract on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85*±0.022 (100.00)	4.67*±0.0058 (96.28)	4.62*±0.0058 (95.26)	4.59*±0.0088 (94.63)	4.48*±0.0375 (92.37)	4.28*±0.0346 (88.25)	4.31*±0.0546 (88.87)	4.036*±0.0218 (83.22)	4.14*±0.0546 (85.36)
ALP	1.25±0.0057 (100.00)	1.243*±0.0029 (99.44)	1.234*±0.0023 (98.72)	1.224*±0.0023 (97.92)	1.217*±0.0041 (97.36)	1.194*±0.0035 (95.52)	1.179*±0.0029 (94.32)	1.146*±0.0061 (91.68)	1.132*±0.0027 (90.56)
LDH	14.59±0.039 (100.00)	14.55*±0.0176 (99.73)	14.21*±0.015 (97.40)	14.44*±0.0218 (98.97)	14.10*±0.042 (96.64)	14.63*±0.0185 (100.27)	14.10*±0.043 (95.89)	14.246*±0.012 (97.64)	13.91*±0.0208 (95.34)
GPT	6.55±0.015 (100.00)	6.53*±0.053 (99.69)	6.37*±0.0176 (97.25)	6.42*±0.029 (98.02)	6.31*±0.02 (96.33)	6.64*±0.0317 (101.37)	6.1*±0.0176 (93.13)	6.19*±0.034 (94.50)	5.97*±0.021 (91.15)
GOT	1.64±0.023 (100.00)	1.75*±0.0176 (106.70)	1.4*±0.0425 (85.37)	1.66*±0.012 (101.22)	1.16*±0.024 (70.73)	1.56*±0.0185 (95.12)	1.04*±0.02 (63.41)	1.464*±0.0208 (89.27)	0.99*2±0.0033 (60.48)

Similarly 40% and 80% of LD_{50} of C-CuT mixture caused a significant ($p < 0.05$) decrease in acid phosphatase (81.38% and 78.52%), alkaline phosphatase (84.48%and 78.59%), lactic dehydrogenase (90.12% and 84.03%), glutamate pyruvate transaminase (86.68% and 80.56%), glutamate oxaloacetate transaminase (80.85% and 76.44%) and acetyl cholinesterase (76.72% and 70.72%) levels after 16 h of treatment in comparison to control respectively (Table 11).

40% and 80% of LD_{50} of *C. decidua* aqueous extract caused significant ($p < 0.05$) decrease in acid phosphatase (83.22% and 85.36%) and alkaline phosphatase level (95.52% and 94.32%) after 16 h of treatment. Contrary to this Lactic dehydrogenase level was found to be increased after 12 h treatment of termites with *C. decidua* aqueous extract. Same extract has also shown slight variation in glutamate pyruvate transaminase level at different treatement in comparison to control (Table 12). Similarly 40% and 80% of LD_{50} of Capparis decidua aqueous extract caused a significant decrease in glutamate oxaloacetate transaminase and acetyl cholinesterase level at all the test time interval and the level recorded 89.27% and 60.48% and 83.01% and 74.31% after 16 h treatment in comparison to control respectively (Table 12).

4 Discussion

Plant products are used as pesticides to kill insect pests. However, in the present study efforts have been made to explore insecticidal potential of *C. decidua* and its mixtures. In various bioassays *C. decidua* extract and its mixtures were found highly toxic which is proved by low LD_{50} value obtained i.e. ranged from 1.56 to 26.0 μg/g body weight after 16 h treatment. Similarly neem bark and cedar wood Douglas fir wood [22, 23] and black heartwood of *Cryptomeria japonica abs* red cedar, Tectona grandis have shown good termiticidal activity against *Coptotermes formosanus* Shiraki [10]. Besides this, limonoids such as obacunone (113 ppm), nomillin (4475 ppm) and azadirachtin (65,293 ppm) exhibited very high toxicity against R. speratus [24]. Similarly essential oils like vetiver grass, Cassia leaf, clove bud, cedar wood, Eucalyptus, lemon grass, geranium were also found highly effective against termites at a very low dose of 1% (W/V). Similarly, anti-termite activity is reported in *Curcuma longa* rhizome oil against *O. obesus* at 2,000ppm concentration [25].

Besides showing toxicity, various extracts have significantly altered the level of various macromolecules and enzymes in treated termites. However, 40% and 80% of LD_{50} of each mixture i. e. C-ST, C-CoT, C-CuT caused significant ($p < 0.05$) reduction in glycogen, amino acid, DNA, RNA, protein and lipid

contents in termites after 16 h of treatment. C-ST mixtureshas decreased lipid contents up to 78.58% and 75.71% after 16 h treatment respectively (Table 1) while 40% and 80% of LD_{50} of *C. decidua* mixed with borate and other natural components caused a significant ($p < 0.05$) decrease in glycogen, amino acid, DNA, RNA, protein and lipid level in termites (Table 2). Similarly *C. decidua* mixed with copper sulphate (C-CoT) mixture has shown significant ($p < 0.05$) decrease in the level of different macromolecules (Table 3). Similar results were obtained in photo-activated cow urine and its combinatorial mixtures with *C. decidua* (Table 4 and 5). It was mostly found that on an average *C. decidua* and its combinatorial mixtures have shown higher toxicity in comparison to other mixture. Morespecifically, termites treated with 40% and 80% of LD_{50} of *C. decidua* aqueous extract showed reduction in glycogen, amino acid, DNA, RNA and protein levels at regular time intervals (Table 6). after response to stress caused by pesticide treatment

In insects, glycogen is a major energy reserve found in fat body and muscles. It is synthesized from glucose units but indirectly it is also synthesized from glucogenic amino acids which indicate utilization of amino acids. Normally free glycogen floats in the haemolymph/blood. Its breakdown or help to maintain glucose level in insect tissues [26]. With this, its utilization exceeds to cope up the insecticide-induced stress [27, 28]. Therefore, phosphorylation of energy molecules is increased which indicates major utilization of food reserves and release of high energy in insect tissues. Therefore, more and more utilization of energy reserves occurs to fight against pesticides generated stress.

Similarly cypermethrin affect the level glycogen level in *Pimpla turionella* wasp larvae, pupae and adult female after treatment [29]. It also increased the protein level in *Spodoptera litrua* larvae [30]. Contrary to this, organophosphorus insecticides i. e. chloropyrifos, thiamethoxam, fipronil, and malathion caused significant depletion of total protein in haemolymph and fat body of silk worm *Bombyx mori* [31]. Similarly, lipid depletion occurs in haemolymph, fat body and oocytes of *Tenebrio molitor* after malathion treatment [32]. It indicates excess utilization of lipids. Hence, to maintain the level of lipids large portions of absorbed carbohydrates are converted into lipids [33]. It may be due to breakdown of glycerides and diglycerides. As insects required lipids was essential dietary constituents if anyhow lipid metabolism is exceeded more then it indirectly cut down carbohydrate reserves. Usually in insects, fatty acids are accumulated in fat body as triglycerides, which serve as energy reserves. Hence, lipid reserves are built up during active feeding. On the other hand mobilization of more lipids may induce hydrolysis of triglycerides and diglycerides by an enzyme lipase. If lipid reserve increases, it means hydrolytic

enzymes are not functioning well and over deposition of lipid may cause oxidative stress in insects. Similarly, excessive utilization of protein and nucleic acids caused physiological stress that result into low availability of these nutrients. Moreover, protein catabolism results in an increase in amino acid. Further, *in vivo* exposure of 40% and 80% of LD_{50} of C-ST, C-BT, C- CoT, PCU, C-CuT and aqueous extract of *C. decidua* caused significant ($p < 0.05$) reduction in the level of acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamatepyruvate transaminase, glutamateoxaloacetate transaminase and acetyl cholinesterase levels (Table 7-12). Similarly solvent and aqueous extracts of *Gloriosa superba* [34], *Paronia emodi* [35], *Corydalis incise* [36], *Cassia obtusifolia* [37], *Artemisia annua* [38], *Teucrium royleanum* [39], *Andrache cardifolia* [40], *Angelica archangelica* and *Geranium sylvatica* caused significant inhibition in the level of acetyl cholinesterase, lipoxygenase, urease and alkaline phosphatase, amino transferase of insects [41]. Alkaloids isolated from amaryllidaceae plants significantly inhibited acetylcholinesterase level in insects [42, 43].

Similarly, *C. decidua* extract and its mixtures significantly inhibited the level of ACP and ALP. It may be caused due to induction of hydrolytic activities to fight against toxic effect of pesticide that lead to significant reduction in the level of acid and alkaline phosphatase. Besides this, alkaline phosphatase level might be increased due to very high lysosomal activity in cells, which leads to biochemical stress in insects [44]. In addition to it pesticide intoxication increase transamination activity that affected the level of glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) in treated termites [45]. However, fat body and heamolymph exhibit higher glutamate oxaloacetate transaminase activity than the glutamate pyruvate transaminase. Hence, the level of heamolymph aminotransferase get significantly decreased. Similarly an increase in glycogenesis causes a significant decrease in free amino acid level [46]. Therefore, a sharp decrease or increase in the level of above enzymes effect oxygen consumption in insects. However, inhibition of phosphatase activity and increase in lactic dehydrogenase level shows tissue necrosis in insects [47]. However, this imbalance in enzyme level indicates inhibition of important metabolic pathways [48]. Similar effects on phosphatases activity were observed in *Pectinophora gossypiella* (Saund.) by Abdel-Hafez et al. [49] after insecticide treatment. Hence, all significant changes in the level of ALP, ACP, GPT, GOT, LDH and AchE indicate very high insecticidal activity of above mixtures to the *O. obesus* the Indian white termite. However, it can be concluded that *C. decidua* possess few active ingredients that might be highly effective against termites. It is proved by the

results that these ingredients cause high lethality in termites at a very low dose and caused significant inhibition or induction of metabolic enzymes. Therefore, it is recommended that *C. decidua* active ingredients could be used for preparation of formulation to control field termites.

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