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MACROFUNGI FLORA OF BEYTEPE CAMPUS AREA (ANKARA)

Perihan GÜLER[1] and Birol MUTLU[2]

Received 04.02.2003

Abstract

In this study, the fungi were collected from Beytepe Campus area in the years of 1999 and 2000, particularly autumn and spring. As a result of these field and laboratory studies, 17 macrofungi were identified, 3 of which belonged to *Ascomycetes* and 14 to *Basidiomycetes*.

Key Words: Beytepe, fungi, taxonomy, mycoflora

Introduction

While much research has been done on macrofungi (1), studies on Turkish macrofungi flora are still incomplete. The aim of this study was to determine the macrofungi taxa of Campus area. Campus workers were collecting these undefined fungi in every year during spring months. There are some poisonous fungi species, which are not known too much can cause lots of danger between the collectors. This area has never studied so far. There isn't adequate knowledge about macro fungal flora of this area.

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The research area is located of the Ankara city in Çankaya district. The area is situated in Irano-Turanian phytogeographical region and B4 square according to Davis' in the years of grid system (2). The area is surrounded to the north by Toprak-Su Damp Lake and trial agriculture areas, to the south by Lodumlu Village and Ankara Cement Factory stone reserves, to the west by Ümitköy - Çayyolu settlement location and several cooperative lands and to the east by Bilkent University Campus area (Figure 1).

The area under review has a length of 6 km in north-south and 4 km in east-west directions. Total area is 5.877.628 km^2. The study area is naturally covered by steppes and rarely *Pyrus elaeagnifolia* Pallas, *Elaeagnus angustifolia* L., *Jasmimum fruticans* L., *Crataegus orientalis* Pallas ex M.Bieb., *Rosa canina* L. trees and shrubs observed in some district (3). This natural composition of the area has lost day by day because of *Pinus nigra* Arn. ssp. *pallasiana* (Lamb.) Holmboe and *Cedrus libani* A.Richard trees have planted in the most of this area.

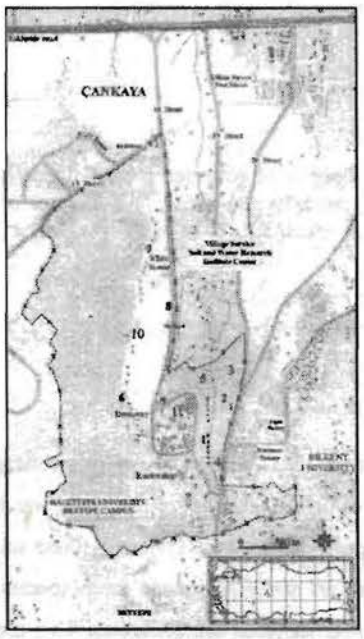

Figure 1. Collected sites of macrofungi in the study area

The annual average rainfall is 13.18 mm and average warmth is 11.29 ^{0}C in the area. The rainfall regime of the study area is "spring-winter-autumn-summer". The coldest month is January with a mean temperature of –21.5 ^{0}C. The dry period is between the end of May and the end of October. With respect to Emberger' s climatic divisions modified for Turkey by Akman (4), the area belongs to the East Mediterranean zone with semi-xeric winters.

Material and Method

The macrofungi specimens of this study were collected in Beytepe Campus area of Hacettepe University in the years of 1999 and 2000. The ecological and morphological characteristics of the macrofungi were recorded and they were photographed in their natural habitats. Specimens were brought to the laboratory and dried after spore prints were obtained. The identification of taxa was carried out according to the literature (5-15). Fungus names, authors, locality numbers, habitats, collecting dates, collector's names and collector numbers were given in floristic list, respectively. The samples are stored at Kırıkkale University Laboratory. Collecting localities in the study area (Figure 1) as in follows:

1.Slopes of Green Valley
2.Stream of Green Valley
3.Northern side of Green Valley
4.Southern side of Green Valley
5.Back of Foreign Languages Higher School, near road
6.Western slopes of student dormitory
7.Back of Biology Department, side of car park
8.Throughout the road
9.Around of white house
10.Between the student dormitory and the white house
11.Between the library and Biology Department

Results and Discussion

The species identified from the research area are listed below.

ASCOMYCETES

Pezizaceae

1-*Peziza badia* Pers. : Fr.

1, 3, 4, 6, 7 on the ground under conifers, 18.03.2000, P.Güler 302-B.Mutlu.

Morchellaceae

2- *Mitrophora semilibera* DC. : Fr.

5, on the ground open woods, near oak, 24.03.2000, P.Güler 352-B.Mutlu.

Helvellaceae

3- *Helvella acetabulum* (L. : Fr.) Quèl.

4, 6, on the ground in open places, 18.03.2000, P.Güler 114-B.Mutlu.

BASIDIOMYCETES

Tricholomataceae

4- *Marasmius oreades* (Bolt. : Fr.) Fr.

8, in grassy areas, often in fairy rings, 24.03.2000, P.Güler 362-B.Mutlu.

Agaricaceae

5- *Agaricus arvensis* Schaeff. : Fr.

5, in fields and grassy area, 23.10.1999, P.Güler 185-B.Mutlu.

Coprinaceae

6-*Coprinus comatus* (Müll. : Fr.) S F Gray

1, 4, very common, in grass and hard packed soil, 16.09.1999, P.Güler 228-B.Mutlu; 24.03.2000, P.Güler 408-B.Mutlu.

7- *Coprinus micaceus* (Bull.: Fr.) Fr.

3, on the ground under pine near stumps, 24.03.2000, P.Güler 296-B.Mutlu.

Cortinariaceae

8- *Inocybe geophylla* (Sow. : Fr.) Kummer,

4, 6,under coniferous plants, 16.09.1999, P.Güler 252-B.Mutlu.

9- *Inocybe maculata* Boud.

4, 6, under coniferous plants, 23.09.1999, P.Güler 111-B.Mutlu.

Pleurotaceae

10- *Pleurotus ostreatus* (Jacq. : Fr.) Kummer.

2, 6, on willow, 18.03.2000, P.Güler 145-B.Mutlu, 23.09.2000, P.Güler 411-B.Mutlu.

Boletaceae

11- *Suillus luteus* (L.) S F Gray

4, 6 under pines,11.09.1999, P.Güler 115-B.Mutlu.

12- *Boletus cavipes* Opat.

3, under pinus, 16.09.1999, P.Güler 132-B.Mutlu.

Sclerodermataceae

13.-*Scleroderma citrinum* Pers.

3, on the ground near trees, 16.09.1999, P.Güler 117-B.Mutlu.

Polyporaceae

14- *Phellinus chrysoloma* (Fr.) Donk.

6, upper pinus, 18.03.1999, P.Güler 402-B.Mutlu.

15- *Trametes versicolor* (Fr.) Pil.

11, on wood, 17.10.1999, P.Güler 377-B.Mutlu.

Lycoperdaceae

16- *Calvatia gigantea* (Bat. : Pers.) Llyod

9, single, in grassy areas, 23.10. 1999, P.Güler 333-B.Mutlu.

Astraeceae

17- *Astraeus hygrometricus* (Pers.) Morg.

10, in groups, in sandy soil, 16,09.1999, P.Güler 366-B.Mutlu.

In this study, 17 macrofungi were identified, 3 of which belonged to *Ascomycetes* and 14 to *Basidiomycetes*. According to the result of this study, 2 species (*Peziza badia* and *Mitrophora semilibera*) are edible and the edibility of one species (*Helvella acetabulum*) is unknown in the *Ascomycetes*. On the other hand, 3 species (*Inocybe geophylla, I.maculata* and *Scleroderma citrinum*) are poisonous, 3 species (*Phellinus chrysoloma, Astreaus hygrometricus* and *Trametes versicolor)* are inedible because of their structure, taste and odour and the rest of them are edible in the *Basidiomycetes*.

Ten of the 17 macrofungi species found in the area are edible, but only three species *Agaricus arvensis*, *Coprinus comatus* and *Pleurotus ostreatus* are commonly eaten. Three species of the taxa are poisonous, 3 species are inedible and the edibility of one species is unknown. With this study made on the macrofungi. Flora of Beytepe Campus, the macrofungi species of newly investigated area to the Turkish of macrofungi Flora.

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THE OCCURENCE OF *Varroa jacobsoni* (Acari: Varroidae) AND *Acarapis woodi* (Acari: Tarsonemidae) IN ANKARA AND ITS SURROUNDINGS

Aslı ÖZKIRIM* and Nevin KESKİN

Received 25.02.2003

Abstract

In this study, 10 158 adult honey bee samples were collected from 52 villages of 13 towns of Ankara. The external surface of adult honey bees were observed easily for the detection of *V. jacobsoni*. Diagnosis of tracheal mite infestation in honey bees were done by the examination of trachea for the presence of mites. In all samples, *V. jacobsoni* was found at the rate of 96%, 5%, 92% in spring, summer and autumn respectively. *A. woodi* could not be found from the honey bees, but it doesn't mean that lack of infestation.

Key words: *Apis mellifera, Acarapis woodi, Varroa jacobsoni,* tracheal mite .

Introduction

Parasitic mites represent on increasing threat to beekeeping worldwide. The originally balanced host-parasite relationship is no longer a fact when a parasite changes to a newspecies of bees; this is intensified through the increasing international exchange of bees.

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This is how *Varroa jacobsoni*, which leads to no economical damage when it lives with its natural host, *Apis cerana,* as an ectoparasite (1, 2). *V. jacobsoni* can develop in a very short time into a parasite for *Apis mellifera* L., and virtually destroy beekeeping in certain countries, such as Turkey.

The honey bee tracheal mite (HBTM), *Acarapis woodi* (Rennie), is a wide spread endoparasite that is associated with mortality and reduced productivity of colonies of honey bees, *Apis mellifera* L. (3, 4, 5, 6). The honey bee tracheal mite, *Acarapis woodi* lives and reproduces within the prothoracic tracheal tubes of honey bees, *Apis mellifera* L. In *A. woodi* infestations, mostly adult honey bees die and this event is called "winter losses" among the beekeepers. Also, early studies showed a positive association between winter losses and the degree of infestation of colonies that had become naturally infested with *A. woodi* (7, 8). This was the first significant quantitative relationship that had been demonstrated between infestation and a poorer performance of bee colonies (3).

It was expected that *V. jacobsoni* and *A. woodi* would not present the same level of problems in Turkey that it has in Europe and USA. This study was conducted to determine the presence and the quantity of *V. jacobsoni* and *A. woodi* infestations in honey bee colonies in the capital city of Turkey; Ankara and its surroundings.

Materials and Methods

V. jacobsoni adults and nymphs were detected in and collected from capped drone brood cells of *Apis mellifera* L., colonies located at 52 villages of 13 towns of Ankara. The studying area is located in the middle of Turkey, Ankara and its surroundings. While the villages of towns selecting, it was looked out the distance between two villages at least 8-10 km for the geographic isolation of the disease. So we know that the flying capacity of honey bee is 3-4 km. During the study, 10158 adult honey bee samples were also collected from 100 hives in 52 apiaries. The honey bee samples were taken for both *V. jacobsoni* and *A. woodi* investigation. The first field study was done in late March/ early April 1999. 50 honey bees were sampled from each colony in 52 apiaries and preserved in 70% ethyl alcohol. All colonies were resampled in July (summer season) and September (autumn season) of 1999.

It was considered that climatic conditions among 52 villages, because the climate influences brood area and so, one important factor in the development of the mite is the extension of the brood area. Adults and/or nymphal stages of *V. jacobsoni* were located in brood cells. At the start of their eclosion, worker bees were removed with forceps and closely examined to determine the presence of exoparasitic mites, *V. jacobsoni* female. As these are very large mites (ca. 2mm across) attaching to the external surfaces of adult bees (workers,

drones) and to the immature stages in capped brood cells; mostly drone cells were observed (9). The brood cells were also examined to remove the debris and remaining parasites.

Only adult female mites and deutonymph females were considered to determine the parasitic intensity in brood cells, because the protonymph females and males are considerably smaller and do negligible damage, based on studies of weight loss after parasitism (10). Bees were grouped according to the presence of malformations in their wings and abdomen.

Diagnosis of *Acarapis woodi* infestation in honey bees were done by the examination of tracheae for the presence of mites. This technique, described by Delfinado-Bakers was used in this study (11). The technique consists of removing the bee's head and front legs under a dissecting microscope to expose the thoracic muscles and tracheal trunks. Healthy tracheae are noticeably clear and colorless, those infested with mites are discolored or blotchy. The tracheae were then removed and placed in lactic acid on a microslide, covered with a coverslip and examined under a compound microscope (x100 magnification) for the presence of mites. This procedure is recommended for a quick examination of a few samples of bees. When dealing with several large samples of bees collected, a thin transverse section of bee thorax was cut after removing the head and front legs. Cut thoracic sections of several bees (approximately 50 bees) were then placed in a glass dish containing 8% potassium hydroxide for clearing at 80 ^{0}C for 10 minutes. These sections were stained with 1% methylene blue and then washed in distilled water. The aim of stainning was to provide different coloration of parasitic mites and tracheae. Cleared sections (exoskeleton and tracheae) were then examined for symptoms under a dissecting microscope. After that, these tracheae were mounted on a microslide in lactic acid and examined microscopically.

Results

In all samples the exoparasitic mite, *V. jacobsoni* was found at the rate of 96%, 5%, 92% in spring, summer and autumn respectively. According to these levels, 50 colonies for spring, 3 colonies for summer, 48 colonies for autumn were infected with *V. jacobsoni* in 52 apiaries. In observations mostly done in the villages and towns, *V. jacobsoni* infestations have been encountered. The distribution of Varroa mites infestations depending on seasons was summarized in figure.

In *A. woodi* infestations, mostly winter losses occur. This event also indirectly causes the

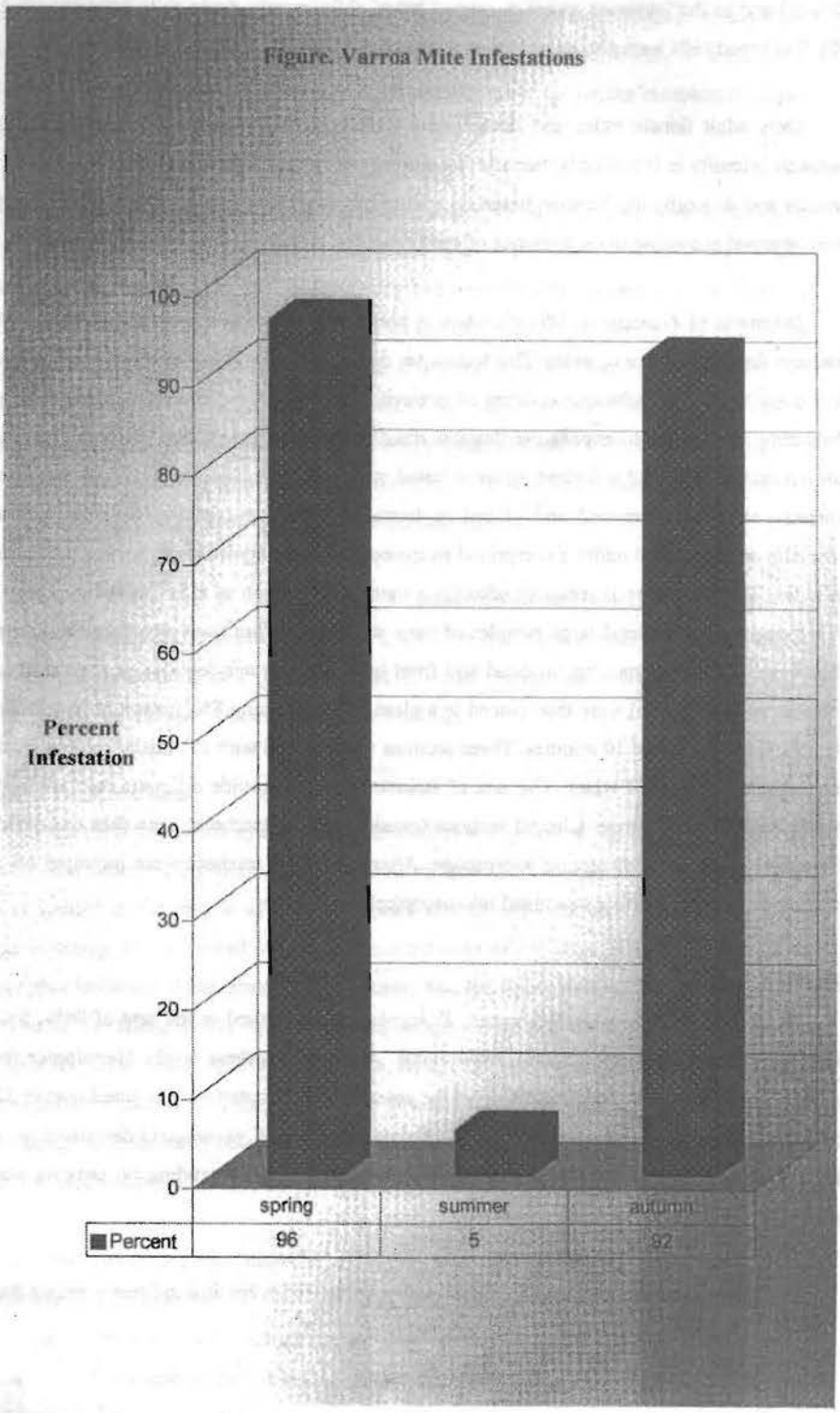
Figure. Varroa Mite Infestations
Percent Infestation
100
90
80
70
60
50
40
30
20
10
0
spring
summer
autumn
Percent
96
5
92

decrease of honey production. According to Pelekassis, *A. woodi* was found in West Trakya region of Turkey (12). This research brings about the possibility of spreading of the disease in the other part of Turkey. In this respect many samples were collected from Ankara and its surroundings but *A. woodi* could not be found from the honey samples.

Discussion

V. jacobsoni is found in all over the world, United States, Papua New Guinea, Indonesia, South Africa, Srilanka etc. (2,9,11). This diseases is wide spread day by day seasonally.

During the autumn months the number of mite females reproduced decreased, with an increase in parasitic intensity. During spring the number of mite males reproduced did not present significant differences with different parasitic intensities. During this season, the number of cells invaded by more than one mite was smaller than for the autumn period. This may be due to the fact that in spring, the queen's greater activity reduces the probability of mites invading cells which have already been invaded. The microclimate and the season of the year may be some of the factors which influence the rate of mite reproduction.

The results of our investigation show a differential reproduction rate during both seasons when the samples were taken. While in spring the percentage was 96%, in autumn it was 92%. In spring, when the brood area is larger, we observed a lower proportion of cells with more than 1 mite and similar proportions of nonreproductive females in cells parasitized by 1 or more mites. So according to Ritter (16) one important factor in teh development of the mite is the extension of the brood area.

The variations in the reproductions levels of *Varroa jacobsoni* may account for a differential growth in the mite populations during the different seasons in temperate climates in Turkey. On the other hand, there is a difference between northern and southern towns' climate of Ankara.

It was observed that the most of the hives were fixed directly on the moistry soil and near the water splashes in the villages. The rapidly growing up grass in spring, covered the hives and conducted the moisture through the hives easily. After the rain, the hives were not aired in order to prevent the brood combs from cold. Thus, every suitable conditions were supplied for growing of the Varroa.

Another significant problem related with the parasitic mite *Varroa jacobsoni*, is improper treatment of the disease in Turkey. For the treatment, many compounds produced by the medicine companies have been tested (13, 14, 15, 16, 17, 18), yet our beekeepers have

been trying to treat honey bees by primitive techniques. They commonly use malathion, flumethrin and also naphtaline. So, Varroa mites get a ressistance to chemical compounds.

Although *Acarapis woodi* was reported in many country, especially Bulgaria, Greece and Russia (19), *Acarapis woodi* infestation couldn't be found in the capital city of Turkey, Ankara and its surroundings but there is still a lot of claim concerning the technique to diagnose *A. woodi*. For instance, because the investigation could be done only in the main tracheae, in lower infestations it is impossible to find out if there is any infestation in minor tracheae (11). Although this method was popular between 1985-1986, today any lower infestations may be diagnosed with chromotographic and molecular techniques (20). Our future research will include these techniques.

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RECENT SITUATION AND POTENTIAL THREATS OF BIRDS OF PREY IN TURKEY

Levent TURAN *

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Abstract

Due to such reasons as its geographical location, present conditions of climate, and the richness of ecosystem, Turkey has a rich fauna. This richness is also reflected in its avifauna. With 454 bird species recorded within its borders, Turkey is one of those countries in the continent of Europe in which the highest number of bird species is observed. Among these species, 40 species are "Diurnal Birds Of Prey"; in addition, 10 nocturnal birds (Owls) are "Nocturnal Birds of Prey ". 37 species of birds of prey, which have various population densities, were recorded in the observations carried out in 2001. These species, which have different status, are faced with various environmental problems of different dimensions.

Key words: Birds of prey, Potential Threats, Turkey.

Introduction

According to (1) the number of the species of the diurnal birds of prey in the world is 284. Raptor species, which live in the Western Palearctic, of which Turkey, too, is a part, reflects small variations according to some researchers. For instance, (2) expresses the number as 42 for Europe and the Middle East; (3) gives the number as 46 species of birds of prey.

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On the other hand, the number of studies in Turkey on birds of prey is rather restricted. On the conjectural density of population of birds of prey in Turkey (4) (5), (6), (7) (8), (9), (10), (11) and (12) on the immigration of birds of prey over Turkey; (13) and (14) on hunting with birds of prey, and (15) on the scales of danger of birds of prey in Turkey have published several studies.

Material and Methods

In this study, the data obtained from our observations, which were carried out at various points representing Turkey's all geographic regions from January 1st through December 31st 2001, and records of literature are taken into consideration (16). The total number of observation as a basis for this consideration is 321. So as to process the data a map divided into squares is made use of in which Turkey Geographical Degree System (UTM) is taken as a basis (see Figure 1.). Resulting 105 squares are each given a symbol consisting of a letter and a number.

In the forms prepared for the total 40 species of birds of prey is recorded how many individuals are observed, in which square and when they are observed. Table 1 is constructed as a result of the analysis of these forms.

Results and Discussion

It is emphasized by (17) and (5) that 39 diurnal species of birds of prey exist in Turkey. One of the lists for bird species, which has most recently been updated is the one prepared by (18), which is Turkey List of Bird Species. 40 out of 454 species given on this list is diurnal birds of prey with various statuses. Their population was observed in miscellaneous density. Table 1 has been constructed by processing 321 observations of diurnal birds of prey. As a result of the evaluation made in line with this Table, the frequency of record of birds of prey in Turkey in 2001 is as follows:

Species Not Recorded in 2001

There is no record whatsoever as to that the species of Oriental Honey Buzzard (*Pernis ptylorynchus)*, Blackshouldered Kite (*Elanus caeruleus*) and *Falco pelegrinoides* are encountered in Turkey in 2001.

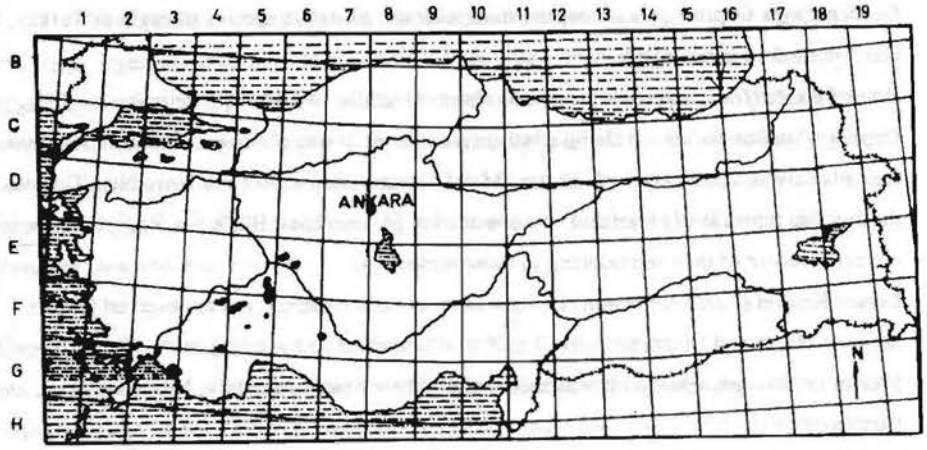
Species Encountered Only Once

Other than these species, the birds of prey observed only once during the one-year observation are, respectively, Red Kite (*Milvus milvus*), Raugh-legged Buzzard (*Buteo lagopus*), and Steppe Eagle (*Aquila nipalensis*).

Species Observed 2 to 5 Times

White-tailed Sea Eagle (*Haliaeetus albicilla*), Bearded Vulture (*Gybaetus barbatus*), Eurasian Griffon Vulture (*Gyps fulvus*), Steppe Harrier (*Circus macrourus*), Levant Sparrowhawk (*Accipiter brevipes*), Imperial Eagle (*Aquila heliaca*), Bonelli's Eagle (*Hieraaetus fasciatus*), Red-footed falcon (*Falco vespertinus*), Sooty Falcon (*Falco concolor*), Lanner Falcon (*Falco biarmicus*) and Saker Falcon (*Falco cherrug*) have emerged as those species observed 2 of 5 times.

Figure 1. Biogeographically regions of Turkey and UTM-based observation squares.



Species Observed Most Frequently

It was identified that the most frequently observed species in 2001 are, respectively, Long-legged Buzzard (*Buteo ruffinus*), Buzzard (*Buteo buteo*), Eurasian Kestrel (*Falco tinnunculus*), the most prevalent kind of Falco species, Marsh Harrier (*Circus aeruginosus*) and Sparrowhawk (*Accipiter nisus*).

Other Birds of Prey

Honey Buzzard (*Pernis apivorus)* It was observed monthly in 7 months between March-September 2001 with a total of 15 times, and one of those observations is the record of an immigration of 550 individuals in August 2001.

Black Kite (*milvus migrans*) is a native species. It was observed 16 times in 11 months in 2001, excluding February. an important one of those observations in the record of 400 individuals in Southeast Anatolia (20).

Egyptian Vulture *(Neophron pecrnopterus*) It was observed monthly in the period between March and September 200, with a total of 13 times.

Black Vulture (*Aegypius monachus*), however, was observed only 4 times in the period of March-October 2001, and recorded 10 times during those 4 months.

Short-toed Eagle (*Circaetus gallicus*) is of those birds of prey encountered relatively frequent. It was recorded totally 23 times in each of the 9 months between February and October 2001.

Hen Harrier (*Circus cyaneus*) is, too, frequently encountered. It is a diurnal bird of prey, recorded in 37 observations carried out between September and April 2001.

Goshawk (*Accipiter gentilis*) was recorded 10 times in 7 months.

Lesser-spotted Eagle (*Aqulia pomarina*) is a species recorded 22 times in 9 months.

Golden Eagle (*Aquila chrysaetos*) the most widespread native species of eagle in Turkey, it was recorded 18 times totally.

Booted Eagle (*Hieraaetus pennatus*) was observed totally 14 times in 8 months.

Osprey (*Pandion haliaetus*) Being a rather rare species, it was observed 7 times in 5 months. Records exist in 2001 only for February, March, August, September and November. Despite the fact that it previously bred and was observed in Marmara and Black Sea Regions, there is no recent record of their reproducing in those regions (4).

Lesser Kestrel (*Falco naumanni*) Being a summer visitor species, it was observed 9 times between March and October.

Merlin (*Falco columbarius*) it was recorded totally 9 times in January, February, April, and December.

Hobby (*Falco* subbuteo) is a species observed 22 times in the 7-month period between April and October.

Eleonorea's Falcon (*Falco eleonorae*) generally having a summer visitor and transit status, this species was recorded 7 times in the 6-month term between April and September.

Red-footed Falcon (*Falco peregrinus*) has a record of 20 times, spreading over 11 months, excluding May.

MAIN FACTORS THREATENING BIRDS OF PREY IN TURKEY

The problems that birds of prey in Turkey and in other countries face show similarities. Other than the large numbers of migratory birds of prey, those species encountered within the borders of Turkey are generally represented by much less individuals that other species of birds. In addition to their small number due to their position in the chain of food, many negative factors, some of which are given below, have been threatening the future of those species. It must be born in mind that problems of population of birds of prey are indicative of serious environmental problems which could have an effect on our lives in various ways.

Habitat Changing (Present or Threatened Risks to Habitat or Range)

Deforestation and other habitat destruction is currently the most serious threat to diurnal birds of prey. Habitat loss not only affects birds in their breeding areas, but also in their wintering areas.

Breeding habitat changing appears to have been probably continues to be greatest threat to viable raptor populations. It is observed that, habitat changing is the probable cause for their population decline. Most of raptors in Turkey utilize extensive, contiguous, mature, wet, hardwood forests; any logging of this habitat is a potential threat.

Pesticides and other Contaminants

Several toxic chemicals and insecticides have been found in some raptors tissues and eggs Eggshell thinning was recorded from many raptor populations. The effect of eggshell thinning on reproductive performance remains inconclusive. Some adult raptors are seen which are died from a combination of some agricultural chemicals. Accidental poisoning occurs when diurnal birds of prey eat rodents or seed-eating birds that have been poisoned by farmers for pest control. Lead poisoning occurs when diurnal birds of prey eat carcasses or live prey shot with lead bullets.

Capture of the Young and Adult Individuals or Egg Collecting

This kind of threat was formerly very common in Turkey, especially in some important bird areas. Though, fortunately, government has initiated an intense training program especially for hunters.

Disturbance (Commercial, Recreational Over-utilization)

The highest sensitivity to disturbance is during nest building and incubation periods. Tree marking, camping, logging, road and dam building, scientific studies are activities that disturb nesting raptors and cause desertion of nests. Nests with young are tolerant to minor human disturbances.

Predation

Predation can be locally heavy on nesting adults, young and eggs. Adults are killed by other larger birds of prey like Golden Eagle (*Aquila chrysaetos*) and Northern Goshawk (*Accipiter gentilis).* They eat a variety of raptors including kestrels, buzzards and kites. Eagle Owls (*Bubo bubo*) are most common predators on eggs and young too. They feed on buzzards, kestrels, kites, goshawks and other owls. In sparrowhawks (*Accipiter nisus*), the larger, mated female will prey on unmated males of the same species that try to court her. Furthermore Common Raven (*Corvus corax*) also destroy eggs or take young. Eggs and young are vulnerable to predation by reptiles and some mammals like porcupine, Red fox

(Vulpes vulpes). Brown Bear *(Ursus arctos)* are suspect for the loss of young at some raptors nests.

Hunting and Other Human-related Factors Affecting Continued Existence of Species

Millions of diurnal birds of prey have been shot in the past. As a group, they have been persecuted for killing livestock, poultry, and game birds. In truth, only a few of the larger species occasionally feed on domestic animals. Some raptor species, were shot just for sport. Though diurnal birds of prey are currently protected in Turkey, some illegal shooting still exists.

Diurnal birds of prey may abandon territories or nest sites if disturbed by human activity. The practice of removing or burning dead livestock (to reduce the risk of disease) has inadvertently reduced the food supply of many scavengers, such as vulture species.

Loss Caused by Vehicles

Many carrion-eating birds,especially vulture species are struck by cars while feeding on or near roads.

Loss Caused by Lines of High Voltage

Accidental deaths occur when birds collide with humanmade structures such as buildings and power lines. Power line electrocution is frequent in larger birds, like vultures and eagles, whose wings can touch two wires at once.

Falconry

In addition to those known problems, it is a known fact that even though it is forbidden, the Sparrowhawks are captured in the Northeast region (13). Falconry is the sport of taking wild game by means of a trained bird of prey, usually a hawk or falcon. Currently, most trained sparrowhawks are used for hunting especially for Quails (*Coturnix coturnix*) . Besides, though not very common, it is documented by the author that some birds of prey are captured for material reasons.

DISCUSSION

Even though there is information in some maps of distribution that the Red Kite species spends the winter in Turkey, this species was observed only once in 2001.

Golden Eagle is the most widespread and crowded population in Turkey, it is pointed out by (4) that it is represented by 2000 to 3000 couples.

Sooty Falcon Despite the fact that it could not be recorded in Turkey in 2001; it was recorded in the previous years in the Southeast region of Turkey.

Black-shoultered Kite is one of those species which could not be recorded in the observations made in 2001. The latest record for this species dates back to 1992.

Falco pelegrinoides was firstly considered to be a new species by (10). It was recorded in several observations in the vicinity of Birecik, Southeast Anatolia, between 1990

and 1994. In the light of these data, it is considered necessary that this species be qualified as "vagrant" or "unknown."

For the purpose of determining the population of Lesser Kestrel in Turkey, a study was carried out in the spring of 1993. According to the findings of this study, (19) stated that the number of Lesser Kestrel couples in Turkey is between 2000 and 3000. In view of these results, it was found out that the population in Turkey is more important than in Spain with which it is compared.

All the species of birds of prey in Turkey are under protection. International Union for the Conservation of Nature (IUCN), a global organization, has been prompting a worldwide tradition of conservation by cooperating with official institutions and voluntary organizations. In the lists of protection written out by this organization, there are 25 species of birds of prey existent in the world.

There are 3 additional lists of CITES Convention, which regulates the international trade of wild animals and plants. Those species in **Appendix-1** are the ones faced with the danger of extinction, and the trading of these is allowed only in exceptional cases. 14 species of diurnal birds of prey are on this list. **Appendix-2** includes those species which are not faced with the danger of extinction, and the commerce of these species is under a very strict control. Excluding 5 Nearctic's vulture species, all the other diurnal birds of prey are on this list.

Dangers with which the birds of prey are faced are more or less the same anywhere. The common conclusion is that these species must be conserved effectively. In order to protect a species or population effectively there is a need for a sizeable accumulation of knowledge in relation to that species in such fields as population biology, population genetics and behavioral ecology. Another effective method other than conservation is to breed and release those species the population size of which has dropped below a certain level. There are several successful examples for this.

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Table 1. Species of birds of prey recorded in Turkey so far, their status and situations in 2001.

				M	O	N	T	H	S							
Latin	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Σ**	**Status**	**Obs**	**Imm**
Pernis apivorus			1	1	1	1	3	4	4				**15**	T, SV		550
Pernis ptylorhynchus													-	V, T		
Elanus caeruleus													-	V, T		
Milvus migrans	2		2	2	1	2	2	1	1	1	1	1	**16**	T, SV	400	
Milvus milvus												1	**1**	R, T, WV		
Haliaeetus albicilla		1	1	1			1		1				**5**	R, WV		
Gypaetus barbatus			1			1			1	1			**4**	R		
Neophron percnopterus			1	2	1	3	2	2	1				**12**	SV, T		
Gyps fulvus					1		1	1					**3**	R, WV		
Aegypius monachus			1				1	2	5	1			**10**	R		
Circaetus gallicus		1	1	1	1	2	2	2	8	1			**19**	SV,T,WV		39
Circus aeruginosus	3	4	7	9	4	1	7	5	15	9	2	10	**76**	R, WV		
Circus cyaneus	5	4	5	1					1	4	3	14	**37**	T, WV		
Circus macrourus									3				**3**	T, WV , SV		
Circus pygargus	1		1	1			1	5	4		2		**15**	T, SV		
Accipiter gentiles	1		1	1		1		1	4	1			**10**	R		
Accipiter nisus	8	5	9	2	3	2	1	2	4	9	3	10	**58**	R, T, WV	68	
Accipiter brevipes					1		1		3				**5**	SV, T		
Buteo buteo	9	5	13	4	4	3	5	12	12	13	6	14	**100**	R, WV		640
Buteo rufinus	8	4	10	7	6	8	10	14	13	11	3	15	**109**	R, WV	86	
Buteo lagopus												1	**1**	WV		
Aquila pomarina		2	3	2	1	2	1	4	6	1			**22**	SV, T		3424
Aquila clanga	1	1		2	1			2	1				**8**	T,WV ,SV		

Aquila nipalensis							1						**1**	T, SV		
Aquila heliaca	1		1					2	1				**5**	R, WV		
Aquila chrysaetos	1		1		1	3	6		2	2	3		**18**	R		
Hieraaetus pennatus			1	1		1	5	1	3	1			**13**	SV, T		
Hieraaetus fasciatus			1	2					1			1	**5**	R		
Pandion haliaetus		1	1					1	3		1		**7**	T, SV, WV		
Falco naumanni			1	2	2	2		2	1	1			**12**	SV,T,WV		70
Falco tinnunculus	8	5	8	2	4	5	17	20	8	6		15	**98**	R	50	
Falco vespertinus					1		1	1	2				**5**	T, SV		
Falco columbarius	5	1		1								2	**9**	WV , T		
Falco subbuteo				2	3	1	4	7	3	3			**23**	SV, T		
Falco eleonorae				1	2	1	1	1	1				**6**	SV, T		
Falco concolor							1		1				**2**	V		
Falco biarmicus							1	1		1			**3**	R		
Falco cherrug						1	1		1				**3**	R		
Falco peregrinus	1	2	2	1		1	2	1	4	4	1	1	**20**	R		
Falco pelegrinoides													-	T, SV		

Explanation of symbols in Table 1

R = Native
SV = Summer Visitor
WV = Winter Visitor
T = Transit
V = Vagrant
Obs. = the highest number obtained during observations
Imm. = the highest number obtained during immigration
$\sum$ = Total number of observation

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THE DETECTION OF THE GENOTOXIC EFFECTS OF SOME NITRO AROMATIC COMPOUNDS BY THE ALLIUM TEST SYSTEM

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Abstract

Three nitroarenes were investigated by using Allium test on the root tips of *Allium cepa* L. Root tips were treated with different series of concentrations of 4- nitro-o-phenylenediamine, 2,4-diaminophenoldihydrochloride and 4-nitroquinoline-1- oxide (4-NQO) ranging from $6.5x10^{-3}$ - $6.5x\ 10^{-8}$ M, $5x10^{-3}$ - $5x10^{-8}$ M and $1x10^{-3}$ - $1x10^{-12}$ M respectively. Used nitroarenes caused decrease in the Mitotic index (MI) and increase frequency of abnormal mitosis when compared with the control.

Key Words: Nitroarenes, Allium test, chromosome aberration

Introduction

Nitroarenes are widespread in the environment because these chemicals are readily formed from products of incomplete combustion of monocyclic and polycyclic aromatic hydrocarbons and oxides of nitrogen. Numerous nitroarenes have been found to induce gene mutation in bacteria and mammalian cells and to induce genotoxicity (induce unseheduled DNA synthesis, single chain scissions and DNA crosslinks, chromosomal aberrations as well as sister chromatid exchange in a variety culture cells) in mammalian

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cells (Nachtman and Wolff, 1982; Neal and Probst, 1983). There are many reports on the carcinogenicity of nitroarenes. The incidence of tumors caused by nitroarenes is high, and in experiments using oral administration of some nitroarenes, bladder, mammary gland and intestinal tumors have been observed in the animals used (Hirose et.al., 1984; Tokiwa and Ohnishi, 1986).

Also numerous nitroarenes are known to cause mutations in the Ames test system (Rosenkranz and Mermelstein,1985; Tokiwa and Ohnishi, 1986; Nakagawa et al., 1987), umu test system (Yoshimitsu et al., 1993; Öztürk and Durusoy, 1999) and to induce genotoxicity in mammalian cell culture system Takayama et. al., 1983; Fifer et al., 1986; Matsuoka et al.,1991). Thus the assesment of their potential risk to humans is of great importance.

Plant assays also have been used as an efficient and reliable test systems for the rapid screening of chemicals for mutagenicity and clastogenicity. Plant testers are relatively cheaper and can easily be stored and handled. Due to the size of their chromosomes, higher plants are suitable for cytological analysis and they show good correlation with other bio-testing systems (Fiskesjö, 1985; Ruiz et al., 1992; Rencüzoğulları et al., 2001). There is a limited number of studies on the determination of genotoxicity of nitroarenes by using plant assays and hence we wanted to evaluate genotoxic potential of monocyclic 4-nitro-o-phenylenediamine, 2,4-diaminophenoldihydrochloride and the well-known genotoxic nitro compound 4-NQO (which is used as a positive control group in this study) by using Allium test system.

Material and Methods

The test chemicals used in this study were obtained from the following sources: 4-nitro-o-phenylenediamine (CAS NO: 99-56-9; purity > 98%) from Merck (USA), 2,4-diaminophenoldihydrochloride (CAS NO: 137-09-7; purity > 97%) from BDH (Germany) and 4-NQO (CAS No: 55-57-5; purity > 97%) from Sigma (USA). The tested chemicals are dissolved in DMSO, therefore the control solution contains DMSO. The amount of test compounds to be used in the experiment were selected based on the preliminary cytotoxicity assays (Dean et al., 1985). According to this assay, the chemicals dissolved at their maximum solubility level and this solution accepted as stock. Concentrations range of test chemicals used in the experiment are prepared by dilution 10 times of stock. The concentrations that are not genotoxic are not used in the experiments. Equal size bulbs of *Allium cepa* L were germinated in tubes containing 10 ml distilled water at 20 ^{0}C. When

the roots reached 1.5 - 2 cm, they were treated with 10 ml test chemicals at concentration of 6.5 10^{-3} x - 6.5x10^{-8} M 4-nitro-o-phenylenediamine, 5 x10^{-3} - 5x10^{-8} M 2,4-diaminophenoldihydrochloride and 1 x 10^{-3} - 1x 10^{-12} M 4-NQO respectively for 3, 6, 12, 24 and 48 hours. At the end of the exposure times, roots tips were fixed in Carnoy's fixative and maintained in 80 % ethyl alcohol. This root tips were prepared using Feulgen's squash technique (Elçi, 1994). According this technique the root tips were hydrolysed in 1N HCl at 60 ^{0}C for 12 min, and finally they were stained in Feulgen for 1 hour. Permanent slides were prepared by the use of alcohol evaporation and were mounted in Canada balsam. These slides were observed under a light microscope (Leitz-Wetzlar). The following microscopic parameters were calculated; i) *mitotic index* (MI), expressed as the ratio of the number of the dividing cells to the total number of cells, and ii) *characterisation of mitosis*, expressed as the number of cells at prophase, metaphase, anaphase and telophase. Three onions were exposed per each treatment and scoring was made from the 3 roots of each replicates. For each concentration of treatment and control, total 2000- 12000 cells were scored. Data were analysed by one-way analysis of variance using ANOVA. For each time period the differences in the means at mitotic index, frequencies of cells with mitotic aberrations, compared to the controls and were evaluated statistically at $p \leq 0.05$ level by Dunnet's multiple comparison test (post-hoc test). Statistical analyses were done angular transformed percentage data (Sokal and Rohlf, 1995).

Results and Discussion

Table 1, 2 and 3 show the MI and the ratio of mitosis stages in the root tip cells of *A.cepa* treated with 4-nitro-o-phenylenediamine, 2,4-diaminophenoldihydrochloride and 4-NQO respectively. All used nitroarenes decreased MI which are statistically significant ($p \leq 0.05$) when compared to the control. The highest decrease in the MI is determined in roots treated with high concentrations and/or prolonged periods of treatments. Decreasing in MI indicates mitotic inhibition and increased frequency of abnormal mitosis. Such 'a decrease in the mitotic index indicates that these chemicals interfere in the normal sequence of mitosis thus preventing a number of cells entering the prophase. Because of the mitotic inhibition by nitroarenes can be suggested blocking of mitotic cycle during interphase which may result from prolonged G_2 period or to the inhibition of DNA synthesis. Used nitroarenes exerted a marked mitodepressive action on mitosis and induced the chromosomal aberrations. The major mitotic abnormalities found at the stages of metaphase and anaphase. The type of abnormalities induced in metaphase

are stickness and c-mitosis and in anaphase are bridges and rarely disturb chromosomes (Table 4-6).

The degree of cytological aberrations in mitosis is regarded as one of the dependable criteria for estimating the effects of mutagen (Reddi and Reddi, 1985). In the present study a remarkable correlation exits between stickness and bridges produced. It was reported that chromosome breaks are highly related to the mutagenic events in the cell (De Serres, 1978).

Table 1. The MI and the ratio of mitosis stages (%) in the root tip cells of *A.cepa* treated with 4-nitro-o-phenylenediamine. *

Conc. (M)	Treatm. Times (hours)	MI ±SD	Prophase ±SD	Metaphase ±SD	Anaphase ±SD	Telophase ±SD
Control		12.0±1.32	75.4±0.3	11.1±0.3	6.0±0.3	7.5±0.3
6.5×10^{-8}		11.5±1.41 **ns**	36.7±0.45	28.3±0.3	27.6±0.35	7.4±0.3 **ns**
6.5×10^{-7}		9.5±1.05	89.8±0.85 **ns**	0.0±0.0	4.7±0.4 **ns**	5.5±0.45
6.5×10^{-6}	3	9.4±0.53	82.5±0.65 **ns**	9.1±0.65	6.5±0.65 **ns**	2.0±0.0
6.5×10^{-5}		7.1±1.17	67.5±0.65 **ns**	13.4±0.65	11.5±1.25	7.6±0.65 **ns**
6.5×10^{-4}		6.3±0.61	51.3±0.9	20.9±1.75	24.4±0.45	3.5±0.9
6.5×10^{-3}		5.5±0.62	47.7±1.5	20.0±0.8	26.2±1.55	6.2±1.60 **ns**
6.5×10^{-8}		8.4±0.53	37.0±0.75	39.1±1.45	17±0.75	6.9±0.4 **ns**
6.5×10^{-7}		7.6±0.60	49.5±2.1	40.0±1.05	3.2±1.05	7.4±1.05 **ns**
6.5×10^{-6}	6	6.8±0.40	62.0±2.25	24.8±1.8	9.7±0.45	3.5±0.0
6.5×10^{-5}		5.9±0.35	62.1±0.75	24.5±0.75	8.4±0.0	5.0±0.4
6.5×10^{-4}		4.0±0.30	51.0±3.4	23.1±1.01	21.8±1.35	4.1±0.7
6.5×10^{-3}		3.7±0.56	48.4±0.81	29.4±1.05	16.3±1.35	6.0±0.0
6.5×10^{-8}		8.9±0.64	72.5±0.9	18.0±0.3	9.6±0.3	0.0±0.0
6.5×10^{-7}		8.2±0.85	57.4±0.35	16.2±0.15	20.1±0.2	6.3±0.55 **ns**
6.5×10^{-6}	12	7.6±0.92	49.5±1.05	40.0±0.5	3.2±0.55	7.3±2.1 **ns**
6.5×10^{-5}		6.3±0.75	33.8±0.35	47.3±1.05	15.7±1.45	3.2±0.35
6.5×10^{-4}		5.4±0.53	40.0±1.7	46.6±1.65	4.2±0.85 **ns**	9.2±0.85 **ns**
6.5×10^{-3}		2.5±0.44	52.9±2.3	33.3±1.15	4.6±1.15 **ns**	9.2±0.0 **ns**
6.5×10^{-8}		6.1±1.50	35.3±0.6	37.1±0.6	20.0±0.3	7.7±1.15 **ns**
6.5×10^{-7}		6.2±0.56	29.0±0.45	67.5±1.4	2.4±0.3	0.0±0.0
6.5×10^{-6}	24	5.7±0.87	26.2±0.8	66.7±0.8	7.1±0.75 **ns**	0.0±0.0
6.5×10^{-5}		4.9±0.36	49.8±0.5	35.7±1.5	10.1±1.55	4.5±0.0
6.5×10^{-4}		4.2±0.80	3.8±0.95	58.5±1.9	26.4±0.95	11.3±1.9
6.5×10^{-3}		4.2±0.46	48.4±2.1	17.9±1.6	17.9±0.5	15.8±1.05
6.5×10^{-8}		8.2±0.72	32.2±1.55	40.8±0.8	23.5±1.15	3.5±0.4
6.5×10^{-7}		9.3±0.61	33.2±0.9	40.6±0.95	20.7±0.95	5.5±0.95
6.5×10^{-6}	48	7.02±1.00	55.9±0.95	42.7±0.95	1.4±0.5	0.0±0.0
6.5×10^{-5}		7.01±0.99	24.0±1.35	73.3±2.05	0.7±0.0	2.1±0.35
6.5×10^{-4}		4.3±0.40	43.1±1.3	54.9±1.3	0.0±0.0	0.0±0.0
6.5×10^{-3}		4.3±0.89	30.4±1.3	39.9±1.9	24.1±1.25	5.7±0.6

* Each value given in the table is the mean of three replicates. Means are significant at $p \leq 0.05$ level compared to the control.

ns: Differences of means were not significant compared to the control.

Table 2. The MI and the ratio of mitosis stages **(%)** in the root tip cells of *A.cepa* treated with 2,4-diaminophenoldihydrochloride.*

Conc. (M)	Treatm. Times (hours)	MI ±SD	Prophase ±SD	Metaphase ±SD	Anaphase ±SD	Telophase ±SD
Control		11.6±1.22	72.4±0.5	14.6±0.75	10.6±0.45	2.4±0.2
5×10^{-8}		10.8±0.61 **ns**	28.1±0.95	48.2±0.3	10.1±0.4 **ns**	13.6±1.9
5×10^{-7}		8.6±0.72	36.1±3.30	36.0±0.2	17.1±0.65	10.8±0.25
5×10^{-6}	3	8.6±0.53	48.1±1.0	35.3±0.5	11.0±0.81 **ns**	5.6±0.55
5×10^{-5}		7.8±0.63	36.9±1.50	33.5±0.6	24.0±0.75	6.5±0.35
5×10^{-4}		7.3±0.46	27.6±1.85	28.2±0.35	30.9±1.35	13.3±0.25
5×10^{-3}		7.0±0.66	29.7±1.95	46.0±0.3	15.1±0.65	9.2±0.25
5×10^{-8}		10.6±0.76 **ns**	57.6±0.25	32.6±2.0	4.2±0.45	5.6±0.45
5×10^{-7}		7.7±0.70	69.1±2.4	27.3±1.2	0.0±0.0	3.6±0.6
5×10^{-6}	6	7.8±0.53	63.6±2.05	34.9±0.55	0.0±0.0	1.5±0.5 **ns**
5×10^{-5}		7.8±0.20	50.5±0.6	45.4±1.35	2.0±0.15	2.1±0.36 **ns**
5×10^{-4}		7.9±0.69	73.1±0.35 **ns**	11.3±0.75	9.3±0.4	6.4±0.25
5×10^{-3}		7.1±0.26	63.6±0.2	21.0±0.4	0.8±0.15	14.6±0.35
5×10^{-8}		8.4±0.53	25.9±0.25	68.3±1.05	4.3±0.25	1.5±0.5 **ns**
5×10^{-7}		8.1±0.40	41.7±0.4	53.6±0.4	3.6±0.4	1.1±0.2 **ns**
5×10^{-6}	12	9.0±0.70	28.7±0.55	60.2±0.45	7.9±0.3	3.2±0.2 **ns**
5×10^{-5}		7.9±0.27	20.4±0.35	60.0±0.35	5.7±0.2	7.2±0.35
5×10^{-4}		7.5±0.50	45.8±0.45	25.0±0.45	23.1±0.3	6.0±0.15
5×10^{-3}		6.4±0.53	43.5±0.45	34.6±0.3	8.1±0.15	11.5±0.3
5×10^{-8}		9.7±1,31	42.9±0.9	42.7±1.75	0.0±0.0	14.4±0.45
5×10^{-7}		9.4±0.53	53.3±0.45	23.4±0.0	18.7±0.5	4.7±0.45 **ns**
5×10^{-6}	24	8.0±1.00	29.3±1.65	65.8±0.55	2.8±0.55	2.2±0.3 **ns**
5×10^{-5}		7.7±0.63	45.6±3.0	27.4±0.95	18.2±1.55	8.8±0.3
5×10^{-4}		7.2±0.40	52.8±0.45	14.7±0.45 **ns**	19.5±0.9	13.1±0.7
5×10^{-3}		6.1±1.01	62.5±0.45	19.8±0.45	2.8±0.2	14.9±0.25
5×10^{-8}		6.7±0.46	45.6±0.4	46.3±0.75	1.2±0.40	7.0±0.75
5×10^{-7}		5.7±0.40	80.8±1.35 **ns**	19.2±0.9	0.0±0.0	0.0±0.0
5×10^{-6}	48	5.6±0.80	63.2±0.95	23.1±0.65	6.9±0.0	6.9±0.65
5×10^{-5}		6.0±0.20	56.7±0.25	30.6±0.5	5.4±0.25	7.3±0.25
5×10^{-4}		5.4±0.53	47.4±3.61	25.3±1.35	24.0±1.8	2.7±0.45 **ns**
5×10^{-3}		5.0±0.89	66.9±1.3 **ns**	22.0±0.65	4.0±0.65	7.2±0.3

* Each value given in the table is the mean of three replicates. Means are significant at $p \leq 0.05$ level compared to the control.

ns: Differences of means were not significant compared to the control.

Table 3. The MI and the ratio of mitosis stages (%) in the root tip cells of *A.cepa* treated with 4-NQO.*

Conc. (M)	Treatm. Times (hours)	MI ±SD	Prophase ±SD	Metaphase ±SD	Anaphase ±SD	Telophase ±SD
Control		12.0 ±0.9	23.9±0.3	29.2±0.3	27.8±0.62	19.8±0.9
10^{-12}		9.6 ± 0.53	67.6±1.7	22.4±0.25	6.9±0.25	3.1±0.25
10^{-11}		9.4 ± 1.51	50.7±0.9	36.1±0.2	6.1±0.65	7.2±0.45
10^{-10}		9.3 ± 0.61	56.8±1.15	31.8±1.15	4.6±1.15	6.8±1.15
10^{-9}		8.8 ± 0.27	54.4±1.4	27.9±0.35 ns	10.3±0.35	7.4±0.35
10^{-8}	3	8.2 ± 0.40	63.2±1.45	20.6±1.5	11.8±0.75	4.4±1.5
10^{-7}		8.1 ± 0.30	41.1±3.75	36.6±0.75	19.4±1.5	3.0±0.75
10^{-6}		8.4 ± 0.40	40.0±0.8	28.2±0.75 ns	23.5±1.55	8.2±0.0
10^{-5}		8.5 ± 0.50	41.3±2.75	46.8±0.9	11.0±1.8	0.9±0.0
10^{-4}		8.2 ± 0.35	10.6±0.15	56.4±0.9	26.0±0.55 ns	6.3±0.15
10^{-3}		7.9 ± 0.50	35.6±0.3	28.4±2.95 ns	28.7±0.6 ns	7.2±0.6
10^{-12}		8.6 ± 0.63	37.6±1.30	27.0±0.55 ns	21.1±0.60	14.3±1.45
10^{-11}		8.4 ± 0.66	44.7±0.8	39.0±1.15	9.5±0.4	6.8±0.83
10^{-10}		8.4 ± 0.53	43.6±0.8	28.4±1.2 ns	13.6±0.6	14.4±0.4
10^{-9}		8.3 ± 0.27	79.5±2.6	9.70±1.05	8.20±1.00	2.6±0.5
10^{-8}	6	8.2 ± 0.30	31.6±2.0	40.3±0.8	17.8±3.95	10.3±0.4
10^{-7}		7.9 ± 0.44	33.3±1.65	23.5±0.5	24.6±2.70	18.6±2.2 ns
10^{-6}		7.9 ± 0.10	34.8±0.0	37.3±2.45	13.9±6.72	13.9±0.0
10^{-5}		7.1 ± 0.27	44.6±1.25	27.7±1.7 ns	10.8±0.45	16.9±0.4
10^{-4}		7.0 ± 0.20	46.1±1.95	43.5±1.95	4.60±0.6	6.5±1.3
10^{-3}		5.8 ± 0.20	40.6±0.95	36.8±0.5	15.4±0.5	13.4±1.0
10^{-12}		8.9 ± 0.20	59.2±2.05	24.5±0.8	10.2±0.8	6.1±0.4
10^{-11}		8.8 ± 0.20	42.7±1.1	42.7±1.1	0.0±0.0	13.60.45
10^{-10}		8.2 ± 0.30	32.2±0.75	40.8±1.6	23.5±0.4	3.5±0.4
10^{-9}		7.7 ± 0.27	45.6±1.5	27.4±1.97 ns	18.2±0.65	8.8±0.3
10^{-8}	12	6.7 ± 0.30	63.8±3.75	21.0±0.4	7.5±0.4	7.7±0.2
10^{-7}		5.5 ± 0.50	46.3±3.85	46.3±1.95	0.0±0.0	6.2±0.8
10^{-6}		6.0 ± 0.30	56.7±0.25	30.6±1.05	0.8±0.25	7.2±0.55
10^{-5}		5.5 ± 0.50	47.7±1.5	20.0±2.3	26.2±3.05 ns	6.2±0.75
10^{-4}		5.5 ± 0.30	66.9±1.3	22.0±1.0	3.9±0.65	7.2±0.65
10^{-3}		4.3 ± 0.30	30.4±2.65	39.9±1.9	24.1±1.25	12.0±0.65
10^{-12}		8.3 ± 0.76	26.7±1.0	35.1±0.95	25.1±0.3	13.2±0.3
10^{-11}		8.1 ± 0.17	29.1±0.9	26.7±0.3 ns	23.1±0.3	21.0±1.5
10^{-10}		8.1 ± 0.56	23.9±0.3 ns	29.2±0.3 ns	27.1±0.6 ns	19.8±0.85 ns
10^{-9}		8.1 ± 0.17	36.3±2.0	33.9±1.6	16.5±0.4	13.3±1.2
10^{-8}	24	8.0 ± 0.20	48.8±1.0	25.4±1.45	14.8±0.72	11.0±1.45
10^{-7}		7.5 ± 0.36	41.6±1.0	33.5±0.5	11.2±1.0	13.7±1.5
10^{-6}		7.2 ± 0.36	42.6±1.15	28.6±0.7 ns	16.3±1.15	12.6±0.95
10^{-5}		6.3 ± 0.27	58.5±1.75	24.9±0.9	10.5±0.9	6.1±0.9
10^{-4}		6.3 ± 0.36	73.4±0.85	21.8±2.15	1.3±0.0	1.3±0.4
10^{-3}		6.5 ± 0.44	56.9±4.75	22.7±1.92	10.9±1.4	9.5±0.95
10^{-12}		7.8 ± 0.35	44.7±0.65	36.5±1.3	11.3±0.6	0.0±0.0
10^{-11}		7.6 ± 0.40	46.7±1.2	27.5±0.55 ns	6.60±0.6	19.2±1.2 ns
10^{-10}		7.5 ± 0.36	42.3±1.9	54.8±1.9	1.90±0.0	0.96±0.0
10^{-9}		7.3 ± 0.40	49.4±1.0	47.1±1.22	1.20±0.0	2.3±0.4
10^{-8}	48	7.0 ± 0.10	59.2±2.05	25.3±0.8	12.2±0.45	2.9±0.45
10^{-7}		7.1 ± 0.27	44.7±0.65	36.5±1.25	11.3±1.25	7.5±1.25
10^{-6}		7.1 ± 0.10	57.4±2.15	31.9±1.3	8.50±0.85	2.1±0.45
10^{-5}		6.7 ± 0.30	64.6±1.05	27.8±2.5 ns	7.60±1.5	0.0±0.0
10^{-4}		6.1 ± 0.20	61.0±2.6	32.5±3.25	6.50±0.65	0.0±0.0
10^{-3}		5.7 ± 0.30	64.6±3.1	21.5±1.55	13.9±1.55	0.0±0.0

* Each value given in the table is the mean of three replicates. Means are significant at $p \leq 0.05$ level compared to the control.

ns: Differences of means were not significant compared to the control.

Table 4. Frequencies of different types of abnormalities in the root tip cells of *A. cepa* treated with 4-nitro-o-phenylenediamine.*

Conc (M)	Tretm.Times (hours)	% of Metaphase abnormalities c-mitosis ±SD (%)	Cells with sticky chromosomes ±SD (%)	% of Anaphase abnormalities Bridge ±SD (%)	Disturb anaphase ±SD (%)	Abnormal cells ±SD (%)
Control		0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
6.5×10^{-8}		45.6±15.0	28.03±4.05	35.2±3.90	0.0±0.0 ns	19±1.85
6.5×10^{-7}		0.0±0.0 ns	0.0±0.0 ns	63.6±9.1	0.0±0.0 ns	2.9±0.45 ns
6.5×10^{-6}	3	89.3±0.75	10.8±0.8 ns	60.0±10.0	0.0±0.0 ns	12.9±1.30
6.5×10^{-5}		17,3±6.94	82.7±6.94	55.5±5.55	0.0±0.0 ns	19.7±1.25
6.5×10^{-4}		13,87±7.32 ns	81.3±10.85	91.7±8.06	8.3±8.06	44.3±3.94
6.5×10^{-3}		11,6±4.01 ns	88.4±4.01	74.47±7.45	10.8±9.49	42.3±0.0
6.5×10^{-8}		4,03±2.31 ns	21.0±2.36	34.1±4.25	0.0±0.0 ns	15.6±0.86
6.5×10^{-7}		5.3±2.65 ns	29.97±3.11	34.0±4.25	0.0±0.0 ns	22.1±0.28
6.5×10^{-6}	6	53.3±5.10	46.67±5.10	92.47±9.42	0.0±0.0 ns	34.1±0.94
6.5×10^{-5}		8,7±3.23 ns	43.77±3.15	78.8±10.45	9.1±0.0	19.9±0.4
6.5×10^{-4}		16.8±5.90	83.23±5.90	90.6±9.35	0.0±0.0 ns	42.9±1.02
6.5×10^{-3}		8,5±4.10 ns	91.47±4.10	100±0.0	0.0±0.0 ns	45.7±2.45
6.5×10^{-8}		3.3±0.0 ns	30.0±3.3	34.1±2.75	4.17±4.78	9.9±1.05
6.5×10^{-7}		4.54±1.61 ns	28.8±2.19	34.12.11	1.1±1.96	12.5±0.36
6.5×10^{-6}	12	6.13±4.95 ns	67.5±6.76	66.67±16.65	0.0±0.0 ns	31.6±1.6
6.5×10^{-5}		48.1±15.77	41.1±3.77	90.9±6.8	0.0±0.0 ns	47.3±2.15
6.5×10^{-4}		49.9±4.85	50.0±4.85	100±0.0	0.0±0.0 ns	50.0±2.5
6.5×10^{-3}		0±0.0 ns	100±0.0	50±0.0	0.0±0.0 ns	35.6±1.15
6.5×10^{-8}		95.8±0.89	4.23±0.89 ns	100.0±0.0	0.0±0.0 ns	57.1±0.25
6.5×10^{-7}		32.03±0.85	67.97±0.85	100.0±0.0	0.0±0.0 ns	71.1±0.45
6.5×10^{-6}	24	29.3±5.20	70.7±5.20	77.8±11.1	0.0±0.0 ns	72.2±1.6
6.5×10^{-5}		55.4±1.22	44.6±1.22	100.0±0.0	0.0±0.0 ns	45.7±3.0
6.5×10^{-4}		82.8±2.40	17.2±2.40	69.3±1.92	30.7±1.92	84.9±2.8
6.5×10^{-3}		5.9±0.5 ns	94.1±0.5	87.3±2.60	12.7±3.12	35.8±2.1
6.5×10^{-8}		23.1±1.45	76.9±1.45	95±1.60	5±1.60	64.3±0.4
6.5×10^{-7}		28.4±3.4	71.6±3.4	0.0±0.0 ns	0.0±0.0 ns	38.3±1.35
6.5×10^{-6}	48	24.6±1.09	75.00±1.85	0.0±0.0 ns	0.0±0.0 ns	54±1.3
6.5×10^{-5}		25.2±12.09	74.7±12.09	100.0±0.0	0.0±0.0 ns	73.9±2.05
6.5×10^{-4}		24.6±1.89	75.4±1.89	0.0±0.0 ns	0.0±0.0 ns	54.9±1.3
6.5×10^{-3}		46.3±1.01	53.6±1.12	63.2±9.12	15.8±5.3	58.9±4.59

* Each value given in the table is the mean of three replicates. Means are significant at $p \leq 0.05$ level compared to the control.

ns: Differences of means were not significant compared to the control.

Table 5. Frequencies of different types of abnormalities in the root tip cells of *A. cepa* treated with 2,4-diaminophenoldihydrochloride. *

Conc (M)	Tretm.Times (hours)	% of Metaphase abnormalities c-mitosis ±SD (%)	Cells with sticky chromosomes ±SD (%)	% of Anaphase abnormalities Bridge ±SD (%)	Disturb anaphase ±SD (%)	Abnormal cells ±SD (%)
Control		0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
5×10^{-8}		2.0±0.4 ns	98.0±0.4	47.1±1.67	2.9±2.55	53.2±0.1
5×10^{-7}		10.23±4.80	90.5±3.66	59.6±0.65	0.0±0.0 ns	46.2±0.35
5×10^{-6}	3	8.73±2.07 ns	91.3± 2.07	59.1±1.1	0.0±0.0 ns	41.9±0.65
5×10^{-5}		5.67±2.01 ns	94.3±2.01	61.9±2.05	0.0±0.0 ns	48.4±0.1
5×10^{-4}		91.7±4.02	8.27±4.02 ns	89.8±1.1	0.0±0.0 ns	56.0±0.0
5×10^{-3}		3.4±1.81 ns	96.6±1.70	98.2±1.85	0.0±0.0 ns	60.8±0.55
5×10^{-8}		9.5±2.97	16.07±1.61	0.0±0.0 ns	0.0±0.0 ns	8.3±0.45
5×10^{-7}		8.9±2.2	17.7±4.55	0.0±0.0 ns	0.0±0.0 ns	7.3±0.6
5×10^{-6}	6	9.7±2.18	16.4±1.67	0.0±0.0 ns	0.0±0.0 ns	9.1±0.5
5×10^{-5}		3.2±0.66 ns	30.7±1.27	60.0±10.0	0.0±0.0 ns	16.6±0.75
5×10^{-4}		10.3±6.56	77.6±7.70	76.3±6.01	4.0±0.0	17.1±0.15
5×10^{-3}		8.9±4.38	91.1±4.38	100.0±0.0	0.0±0.0 ns	21.8±0.66
5×10^{-8}		62.3±16.65	46.3±3.87	23.5±5.85	0.0±0.0 ns	69.4±1.25
5×10^{-7}		72±2.85	28.1±2.90	23.5±0.0	0.0±0.0 ns	54.5±0.45
5×10^{-6}	12	39.03±3.02	61.0±3.02	71.5±6.56	6.8±0.86	66.4±0.66
5×10^{-5}		7.6±1.20 ns	92.4±1.20	64.5±6.45	19.4±11.64	64.3±0.0
5×10^{-4}		12.5±1.15	87.5±1.15	76.6±1.9	0.0±0.0 ns	42.8±0.85
5×10^{-3}		1.5±0.98 ns	98.5±0.98	66.7±7.10	0.02±0.0 ns	40.2±0.6
5×10^{-8}		13.3±4.05	86.7±4.05	0.0±0.0 ns	0.0±0.0 ns	42.7±1.75
5×10^{-7}		18.7±3.21	81.3±3.21	10.0±1.25	0.0±0.0 ns	25.2±0.25
5×10^{-6}	24	11.6±3.89	88.4±3.89	20.0±0.0	0.0±0.0 ns	66.3±0.55
5×10^{-5}		9.6±2.27	90.4±2.27	50.0±3.3	0.0±0.0 ns	36.5±1.5
5×10^{-4}		45.3±5.39	54.7±5.39	97.3±2.97	0.0±0.0 ns	33.7±1.00
5×10^{-3}		3.1±0.7 ns	96.9±0.7	100.0±0.0	0.0±0.0 ns	22.6±0.2
5×10^{-8}		32.3±13.41	74.1±7.23	0.0±0.0 ns	0.0±0.0 ns	46.3±0.75
5×10^{-7}		90.5±0.45	9.5±0.45	0.0±0.0 ns	0.0±0.0 ns	19.2±0.9
5×10^{-6}	48	16.3±2.87	83.7±2.87	50.0±4.55	0.0±0.0 ns	26.5±0.3
5×10^{-5}		25.5±7.7	74.5±12.40	61.9±4.8	0.0±0.0 ns	33.9±0.75
5×10^{-4}		46.7±2.94	53.3±2.94	88.9±3.7	0.0±0.0 ns	46.7±0.45
5×10^{-3}		0.0±0.0 ns	100.0±0.0	83.3±8.35	0.0±0.0 ns	25.3±0.57

* Each value given in the table is the mean of three replicates. Means are significant at $p \leq 0.05$ level compared to the control.

ns: Differences of means were not significant compared to the control.

Tablo 6. Frequencies of different types of abnormalities in the root tip cells of *A. cepa* treated with 4-NQO.*

Conc (M)	Tretm. Times (hours)	% of Metaphase abnormalities		% of Anaphase abnormalities	Abnormal cells ±SD (%)
		c-mitosis ±SD (%)	Cells with sticky chromosomes ±SD (%)	Bridge ±SD (%)	
Control		0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
10^{-12}		2.5±0.05	97.5±0.05	32.0±4.0	24.7±0.55
10^{-11}		6.6±0.69	93.4±0.69	11.1±3.7 ns	36.8±0.0
10^{-10}		20.3±4.83	79.7±4.83	25.0±0.0	33±1.15
10^{-9}		9.3±2.65	88.2±0.75	13.8±3.45 ns	29.3±0.35
10^{-8}	3	0.0±0.0 ns	7.1±0.0 ns	0.0±0.0 ns	25±0.0
10^{-7}		9.5±1.17	90.5±1.17	92.3±7.7	54.5±0.75
10^{-6}		0.0±0.0 ns	13.0±2.91	15.0±1.7 ns	71±1.2
10^{-5}		7.87±3.52	92.1±3.52	100.0±0.0	57.8±2.75
10^{-4}		6.9±4.14	93.1±4.14	100.0±0.0	82.8±1.42
10^{-3}		0.0±0.0 ns	100±0.0	99.7±0.57	56.9±4.5
10^{-12}		0.0±0.0 ns	100.0±0.0	0.0±0.0 ns	26.9±0.55
10^{-11}		2.9±0.9	97.1±0.9	76.0±4.0	46.2±0.75
10^{-10}		3.9±1.47	96.1±1.47	28.6±2.85	32.3±1.6
10^{-9}		0.0±0.0 ns	100.0±0.0	0.0±0.0 ns	9.7±1.05
10^{-8}	6	12.4±2.70	87.6±2.70	62.2±4.50	51.4±1.6
10^{-7}		5.4±1.21	94.6±1.21	55.6±4.45	37.2±0.55
10^{-6}		6.7±4.97	93.3±4.97	85.0±5.0	49.1±1.75
10^{-5}		5.8±2.60	94.2±2.60	100.0±0.0	38.5±2.15
10^{-4}		6.9±0.66	93.1±0.66	100.0±0.0	48.1±2.6
10^{-3}		14.6±1.97	85.4±1.97	100.0±0.0	52.2±0.0
10^{-12}		15.5±1.53	84.5±1.53	100.0±0.0	34.7±1.6
10^{-11}		12.8±4.42	87.2±4.42	0.0±0.0 ns	42.7±1.1
10^{-10}		8.3±3.83	91.7±3.83	100.0±0.0	64.3±2.05
10^{-9}		6.7±2.08	93.3±2.08	50.0±1.7	36.5±0.9
10^{-8}	12	26.7±3.10	73.3±3.10	100.0±0.0	28.5±0.75
10^{-7}		24.9±6.49	75.1±6.49	0.0±0.0 ns	46.3±1.95
10^{-6}		14.6±2.10	85.4±2.10	57.1±9.55	33.7±0.5
10^{-5}		0.0±0.0 ns	100.0±0.0	82.4±2.95	41.5±1.55
10^{-4}		90.1±0.80	7.0±1.73 ns	83.3±8.35	24.6±0.7
10^{-3}		39.2±7.95	56.14.83	79.0±10.93	57±1.93
10^{-12}		0.0±0.0 ns	100.0±0.0	43.6±2.6	46.0±1.6
10^{-11}		0.0±0.0 ns	100.0±0.0	50.7±1.3	38.4±0.0
10^{-10}		0.0±0.0 ns	100.0±0.0	45.7±2.15	35.3±0.75
10^{-9}		0.0±0.0 ns	100.0±0.0	53.7±4.85	42.7±0.85
10^{-8}	24	0.0±0.0 ns	100.0±0.0	83.9±12.9	37.8±0.5
10^{-7}		6.6±1.73	93.4±1.79	95.5±4.55	44.2±1.0
10^{-6}		0.0±0.0 ns	100.0±0.0	71.4±7.15	40.2±0.45
10^{-5}		5.3±3.26	94.7±3.29	58.3±12.5	31.0±±2.2
10^{-4}		0.0±0.0 ns	10.0±2.0 ns	100.0±0.0	35±0.4
10^{-3}		6.9±0.0	88.8±6.36	100.0±0.0	32.7±2.4
10^{-12}		0.0±0.0 ns	100.0±0.0	88.9±11.1	46.5±2.55
10^{-11}		0.0±0.0 ns	100.0±0.0	27.3±9.1	29.3±1.2
10^{-10}		8.8±0.3	91.2±0.3	100.0±0.0	56.7±1.95
10^{-9}		7.6±2.56	92.4±2.56	100.0±0.0	48.3±1.16
10^{-8}	48	7.5±1.89	92.5±1.89	100.0±0.0	37.6±1.25
10^{-7}		0.0±0.0 ns	100.0±0.0	88.9±5.55	46.5±0.65
10^{-6}		19.1±2.06	80.9±2.06	100.0±0.0	10.4±2.15
10^{-5}		7.1±2.74	92.9±2.74	100.0±0.0	65.4±4.05
10^{-4}		11.4±6.60	86.9±4.45	100.0±0.0	39.0±2.6
10^{-3}		0.00±0.0 ns	100.0±0.0	100.0±0.0	35.4±0.0

* Each value given in the table is the mean of three replicates. Means are significant at $p \leq 0.05$ level compared to the control

ns: Differences of means were not significant compared to the control.

Study of potent mutagenicity and carcinogenecity of nitroarenes has been shown by using various bioassays (Rosenkranz ve Mermelstain, 1983; Tokiwa ve Ohnishi, 1986). The genotoxic and mutagenic potentials of numerous nitroarenes that react directly with DNA are shown to be due to the generation of electrophilic species mediated by the nitroreductase and o- acetyltransferase activities in bacteria (McCoy et al., 1982; Rosenkranz and Mermelstein, 1983; Shane et al., 1991). In our study, 4NQO was found a potential genotoxic in accordance with the study Öztürk and Durusoy (1999) in which 4NQO was a potent inducer in umu test system spesifically developed for screening nitroarenes. Parallel results were obtained with the results of studies mutagenic effects of benzo-a- pyrene on chinese hamster cell line V79 and on allium test (Vig, 1978).

As a conclusion it is suggested that, Allium test system is an efficient and reliable short - term bioassay for rapid screening of genotoxic potential of various nitro-containing chemicals, especially coloured nitroarenes which makes useless the test systems such as SOS-chromotest and umu test which is developted for the screening of the mutagenicity of the nitroarenes.

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DETECTION AND FREQUENCY ANALYSIS OF A SLOWER ELECTROPHORETICAL ALLELE AT *αGPDH* LOCUS IN A NATURAL POPULATION OF *DROSOPHILA MELANOGASTER* FROM TURKEY.

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Abstract

A new allele of *αGpdh* locus was detected in a population *Drosophila melanogaster* from Ankara province of Turkey. This allele (designated S') is slower in mobility than the common Slow (S) allele at *αGpdh* locus and appears in low frequency. A comparison with another locus, *Adh* (alcohol dehydrogenase), from the same individual flies indicates that this lower frequency could be explained less by sampling error (drift).

Key Words: *Drosophila melanogaster*, allozyme, *αGpdh*

Introduction

The determination of level of polymorphisms at allozyme loci has been one of the primary concern for evolutionary genetics studies for the ease with which allozymes provide data of genetic variation in natural populations of almost any species (Lewontin 1974). However, the constraint on allozyme polymorphism in the description of total genetic

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variation in a population seems to make the use of them cause spurious assumptions, a case which becomes entangled especially with loci having a high number of alleles (Barbadilla et al., 1996). The situation is reversed if the loci scrutinized electrophoretically have allelic states corresponding to the states at DNA level (Barbadilla et al., 1996). Thus it is expected that with loci having small number of electrophoretical states (alleles) more realistic inferences about evolutionary forces from allozymes are possible.

Glycerophosphate dehydrogenase (*αGpdh*) locus of *Drosophila melanogaster* is located on the left arm of the 2nd chromosome and has two common alleles (F: Fast and S: Slow) in natural populations (Kamping, 2000). Gpdh shows latitudinal frequency changes but the change is not sharp, for example, as that of *Adh* locus (Kamping, 2000 and references therein). Various selective scenarios have been put for αGpdh ranging from differential allelic contributions in lipid metabolism to different flight output capaties (Kamping, 2000). However, the level of selection on *αGpdh* seems considerably less than on *Adh* (alcohol dehydrogenase) (Kamping and Van Delden, 1999), a locus with which *αGpdh* may have significant gametic disequilibria in natural populations (Van Delden and Kamping, 1997). In this study a rare slower allele (S'; slower than the usual slow,S, allele) is presented as the first record of a natural population from Turkey. Allele frequency analysis of *αGpdh* is also presented with the whole set of alleles (including the rare slower, S') detected in the population sample. Another locus, *Adh*, of substantial importance in combination with *αGpdh* is included in frequency analysis, with the aim of broadening the scope of insight into the gene frequency evolution at *αGpdh*.

Material and Methods

A population sample from Ankara province was collected in September 1999. The wild collected flies were placed in 5 replicates of half pint bottles with standard *Drosophila* medium for egg laying in order to establish a laboratory population at 25 °C. Electrophoresis was carried out with the wild collected flies.

Electrophoresis

Electrophoresis was of the standard PAGE system developed for the combination of the *ADH* and *GPDH* by Van Delden and Kamping (1989). Individual flies were homogenized in demineralized water and 3µl of each homogenate was run on the gel. Running buffer was a mix of 0.0205 M Veronal, 0.003 M EDTA and 0.075 Tris at pH 8.4. Reaction buffer per gel consisted of 400 mg Glycreophosphate, 20 mg NAD+, 20 mg MTT, and 1mg PMS all dissolved in 60 ml of 0.2 M Tris-HCl solution at pH 8.5. After 2.5 hs of running gels were placed in a plastic container containing the reaction buffer and put into an incubator shaker

operated at 30 °C for 10 mins. After the *GPDH* bands had appeared, 200 µl Isopropanol (propan-2-ol) was added into the total mix in the container and the gel allowed for 5 mins in the shaker for the appearance of *ADH* bands. When the gel had been clearly stained for the *Adh* and α*Gpdh* electromorphs, they were photographed for scoring using an image analyzer software.

Statistics

Hardy-Weinberg equilibrium was tested with standard chi^2 (χ^2) for *Adh*. For *αGpdh* the equilibrium assumption was tested using an exact test for multiple alleles. The rationale for this was that there were three alleles detected for that locus with the rare allele (the slower) having genotype numbers less than five (see Weir, 1996 for details).

Table 1. Genotype numbers and allele frequencies at *αGpdh* and *Adh* loci in the study. F and S are for the Fast and Slow mobilities, respectively. S' is for the new, slower allele found in this study.

Genotype	Number		Allelic Frequency		
	Observed	expected	F	S	
Adh^{FF}	58	57.48	**0.782**	**0.218**	
Adh^{FS}	31	32.05			$\chi^2 = 0.0096$ ns
Adh^{SS}	5	4.47			
Total	94	94			
$Gpdh^{FF}$	34	32.72	F	S	S'
$Gpdh^{FS}$	43	41.26	**0.590**	**0.372**	**0.038**
$Gpdh^{SS}$	10	13.01			
$Gpdh^{FS'}$	0.0	4.21			P = 0.0347*
$Gpdh^{SS'}$	7	2.66			
$Gpdh^{S'S'}$	0.0	0.14			

* P< 0.05 ns: nonsignificant

Results and Discussion

Almost a hundred individuals were electrophoresed and the population sample was found to be polymorphic for the Fast (F) and Slow (S) alleles of both *αGpdh* and *Adh* (Table 1). The unique finding is that at *αGpdh* locus, an additional allele of different mobility appeared with a frequency value less than 5 percent (Table 1, S'). This allele has slower

mobility than the usual Slow of *αGpdh* and is shown in Figure 1 within a SS' genotype. Indeed it is the only genotype of this slower (S') allele found in this study, which cause significant deviation from the Hardy-Weinberg equilibrium at *αGpdh* locus (P= 0.0347, Table 1). Whether this exclusive combination of S' allele with usual S was the consequence of a definite microevolutionary process is not known. However, an explanation of genetic drift seems less feasible, for the S' allele was lost from the population in few generations under controlled laboratory conditions (not shown). Therefore, a selection against this rare allele would be more likely to occur. That the deviation from Hardy-Weinberg equilibrium may be caused by a significant sampling error is additionally invalid because of the equilibrium detected at another loci, *Adh*, which comprised the data from the same individual flies as that of *αGpdh* (Table 1).

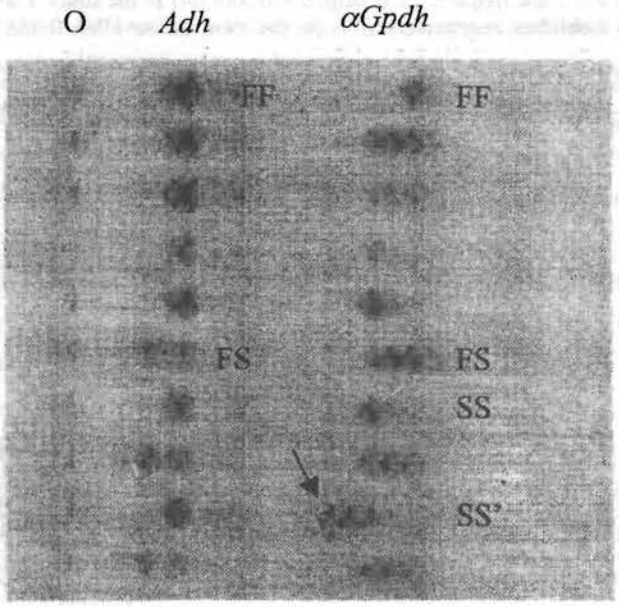

Figure 1. A portion of a gel showing the genotypes at αGpdh and Adh. Arrow indicates the SS' genotype of S' allele detected in the study. O is the origin of individual homogenate loading.

Interestingly, this slower allele has the same electrophoretical mobility with the one described in Kamping and Van Delden (1991). Finally, preliminary DNA sequencing of this Ankara *αGpdh S'* detected in the present study suggests that it may have occurred by gene duplication, which may have led to an allele of quite distinct electrophoretical mobility (ED Ozsoy, L van de Zande and W. van Delden. Unpublished results). Further analyses are necessary to measure the net fitness effect of this rare allele on its carriers, which could be

achieved comprehensively taking the duplicated structure of the allele with the ensuing gene product into consideration.

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A STUDY ON THE DETERMINATION OF INSECT FAUNA IN BEYŞEHİR LAKE RIVER BASIN

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Abstract

In the observations for the determination of insect fauna in the river basin of Beyşehir Lake, 259 species that belong to 76 families have been determined. Among those species, a new subspecies (***Phryganea***: Phryganeidae) for the world of science, and a new record genus (***Barycnemis***: Ichneumonidae) for Turkey have been observed.

Key Words: Beyşehir Lake, insect fauna, new subspecies, new record.

Introduction

The fauna of the basin area were determined comprising the long-term development plan of the Beyşehir Lake river basin. This study is a division of the research mentioned above which includes the insect fauna part. There has not been any studies on insect fauna in Beyşehir lake basin area. According to the data obtained from the literature, some researchers notified some insect species in Beyşehir, when they were observing Konya and Central Anatolia (Aslan et all. 1997, 2003; Çakır and Önder, 1990; Heiss and Önder, 1991; Mergen

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1993; Özbek and van der Zenden, 1992a, 1992b, 1993; Sert, 1995; Tuatay, 1990; Yıldırım and Özbek, 1996; Yıldırım and Kojima, 1999). Since the number of the species is enormous worldwide, the studies on insect fauna is generally done in a limited way by taking only one family or one type. Contrary to other studies, this study aims at collecting all insect species and determining the whole fauna in the area. The collected samples were sent to the area specialists. Some of the groups could not be identified. Some of those groups were identified by the help of keys (Southwood, at all., 1959; Pehlivan, 1981; Lodos, 1984, 1991, 1998) and by using museum materials. The list of species that was obtained from the observation area covers only the species that are identified.

The habitat type and the altitudes have also been added to the species list which is presented in Table 1 depending on the different habitat types that exist in Beyşehir Lake river basin.

This study presented a new subspecies (*Phryganea grandis serti*-Phryganeidae: Trichoptera) for the world of science and a new record (genus) (*Brachynemis*-Ichneumonidae :Hymenoptera) for Turkey. The new species was introduced to the world of science by Sipahiler (2000), however the new recorded genus has not been published by the related specialist yet.

Material and Method

Insect samples were collected during May 1998-July 1999. Insect net and light trap were used in collecting insects. The samples were processed with standard methods and added to the collection. Each group of those samples were identified by the specialists. Both the personal collections (Neşe Çağatay-Lygaeidae, Selim S. Çağlar-different families of Hemiptera) and the samples in the museums (Agricultural Struggle Institute Museum, Hacettepe University Zoology Museum) were examined and identified or they were asked to be identified. Besides, many sources (Lodos, 1984, 1991-1998; Pehlivan, 1981; Southwood and Leston, 1959) were searched thoroughly and common species were identified. The names of the specialists and the groups they identified are as follows: Bruchidae (Coleoptera), by Orhan Mergen; Cerambycidae by Birsen Önalp, Chrysomelidae by Hüseyin Özdikmen and Tuncay Türkeş, Asilidae (Diptera) by Yusuf Durmuş, Tabanidae by A. Yavuz Kılıç, Apidae (Hymenoptera) by A. Murat Aytekin, Brachonidae by Yasemin Güler, Ichneumonidae by Yasemin Özdemir, Lepidoptera, by Selma Seven, Odonata by Ali Demirsoy and Yusuf Durmuş, Orthoptera by Ali Demirsoy and Hasan Sevgili, Trichoptera by Füsun Sipahiler.

Results

In the river basin of Beyşehir Lake specimens in various groups of insects were collected. 259 different species in 76 families have been determined. The taxonomic conditions and ordo levels have been presented in Table 1.

Table 1. List of the Insect Species on Beyşehir Lake Basin

	Locality –Date	Habitat	Altitude
ODONATA			
Coenagriidae			
Ischnura pumilia (Charpentier, 1825)	Beyşehir, 2.7.1998	Land and water lanes	1130 m
Calopterygidae			
Calopteryx splendens splendens (Harris, 1782)	İlıırmak River-Yeşildağ, 2.7.1998	Agricultural area,water lanes	1125 m
Libellulidae			
Crocothemis erythraea (Brulle, 1832)	Near Tolca, 4.7.1998	Land and water lanes	1130 m
Sympetrum sanguineum (Müller, 1764)	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Orthetrum brunneum (Fonscolombe, 1837)	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Gamphus sp.	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
DERMAPTERA			
Forficulidae			
Forficula sp.	Dolama Ridge, 29.5.1998	Forest (Juniper)	1130 m
MANTODEA			
Mantidae			
Ameles syriensis Gigilio-Tos, 1915	İncebel Cape, 29.8.1998	Land and water lanes	1130 m
Mantis religiosa Linnaeus, 1758	Dolama Ridge, 4.7.1998	Forest (Quercus)	1330 m
ORTHOPTERA			
Acrididae			
Acrida bicolour (Thunberg,)	Near Tolca, 4.7.1998	Land and water lanes	1130 m

Acrotylus insbricus (Scopoli, 1786)	Çiftlikköy Locality, 29.8.1998	Agricultural area	1130 m
Chorthippus biguttulus (Linneaus,1758)	Dolama Ridge, 28.5.1999	Forest (Juniper)	1130 m
Chorthippus brunneus (Thunberg,1815)	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Chorthippus dorsatus dichrous (Eversman,1859)	Damla District, 28.8.1998	Forest (Pinus)	1470 m
Chorthippus mollis (Charpentier, 1825)	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Dociostaurus genei (Ocskay, 1833)	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Dociostaurus maroccanus (Thunberg, 1815)	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Euchorthippus sp.	Dolama Ridge, 30.6.1998	Forest (Juniper)	1130 m
Oedipoda aurea Uvarov, 1923	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Oedipoda miniata (Pallas, 1771)	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Oedipoda schochi Saussure, 1884	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Gryllidae			
Oecanthus pellucens (Scopoli, 1763)	Çiftlikköy Locality, 29.8.1998	Agricultural area	1130 m
Gryllotalpidae			
Gryllotalpa gryllotalpa (Linneaus,1758)	Beyşehir, 2.7.1998	Agricultural area	1130 m
Tettigonidae			
Callimenus macrogaster (Lefebvre, 1831)	3 km away from Yeşildağ, 3.7.1998	Forest (Quercus)	1250 m
Decticus verricuvoris (Linneaus,1758)	Kıreli, 28.5.1999	Steppe	1150 m
Platycleis escalaria iranica Ramme, 1929	Near Tolca, 4.7.1998	Land and water lanes	1130 m
Platycleis intermedia (Serville, 1839)	Damla District., 28.8.1998	Forest (Pinus)	1470 m
Platycleis sp.	Çiftlikköy Junction, 28.5.1999	Agricultural area	1130 m

Poecilimon sp.	Çiftlikköy Junction, 28.5.1999	Agricultural area	1130 m
Tettigonia caudata (Charpantier, 1845)	Çiftlikköy Junction, 4.7.1998	Agricultural area	1130 m
HEMİPTERA			
Alydidae			
Camptopus lateralis (Germar, 1817)	3 km away from Yeşildağ, 3.7.1998; Near Tolca, 4.7.1998.	Forest (Quercus); Land and water lanes	1250 m, 1130 m
Coreidae			
Coreus marginatus marginatus (Linnaeus, 1758)	Çiftlikköy Junction, 28.5.1999.	Agricultural area	1130 m
Coriomeris hirticornis (Fabricius, 1794).	Aygır Island, 2.7.1998	Land and water lanes	1130 m
Geocoris Forest (Juniper)i (Herrich-Schäffer, 1839)	Yakamanastırı, 29.5.1998	Forest (Juniper, Quercus, Abies)	1270 m
Lygaeidae			
Lygaeus equistris (Linneaus,1758)	Çiftlikköy Locality, 4.7.1998	Agricultural area	1130 m
Macroplax fasciata (Herrich-Schäffer, 1835)	Dolama Ridge, 29.5.1998	Forest (Juniper)	1130 m
Oxycarenas pallens Herrich-Schaeffer, 1858	Dolama Ridge, 29.5.1998	Forest (Juniper)	1130 m
Piocoris erythrocephalus (Lepeletier et Serville, 1825)	Çiftlikköy Locality, 11.6.1998	Agricultural area	1130 m
Rhyparochromus phoeniceus (Rossi, 1794)	Çiftlikköy Locality, 3.7.1998	Agricultural area	1130 m
Tropidothorax leucopterus Goeze, 1778	Çiftlikköy Locality, 17.10.1998	Agricultural area	1130 m
Miridae			
Brachycoleus decolour Reuter, 1887	Kuşluca, 11.6.1998; Çiftlikköy Locality, 3.7.1998	Agricultural area	1170 m, 1130 m

Calocoris krueperi (Reuter, 1880)	Kurucaova, 3.7.1998	Forest (Pinus)	1470 m
Capsodes cingulatus (Fabricius, 1787)	1 km away from Bekdemir, 10.6.1998	Forest (Quercus)	1330 m
Dryophilocoris sp.	Yakamanastırı, 28.5.1999	Forest (Juniper), Quercus, Abies)	1270 m
Miris sp.	Yakamanastırı, 28.5.1999	Forest (Juniper), Quercus, Abies)	1270 m
Exolygus rugulipennis (Poppius, 1911)	1 km away from Bekdemir, 10.6.1998	Forest (Quercus)	1330 m
Stenodema sp.	Kuşluca, 11.6.1998	Agricultural area	1170 m
Nabidae			
Nabis pseudoferus Remane, 1949	Yakamanastırı, 28.05.1998	Forest (Juniper), Quercus, Abies)	1270 m
Pentatomidae			
Aelia albovittata Stal, 1865	Dolama Ridge, 29.5.1998	Forest (Juniper)	1130 m
Aelia rostrate Boheman, 1852	Bademli road, 28.6.1998	Steppe	1140 m.
Carpocoris pudicus (Poda, 1761)	Dolama Ridge, 30.6.1998	Forest (Juniper)	1130 m
Dolycoris baccarum (Linnaeus, 1758)	Dolama Ridge, 29.5.1998	Forest (Juniper)	1170 m
Eurydema blandum Horv.,	Near Çiftlikköy, 4.7.1998	Agricultural area	1130 m
Eurydema rugulosum (Dohrn.)	Yakamanastırı, 29.5.1998	Forest (Juniper, Quercus, Abies)	1270 m
Palomena sp.	Kurucaova, 30.6.1998	Forest (Pinus)	1500 m
Rhaphigaster nebulosa (Poda, 1761)	Yakamanastırı, 29.5.1998	Forest (Juniper)	1270 m
Staria lunata (Hahn, 1835)	Karaburun-Gavur Reed Bed, 31.5.1998	Land and water lanes	1130 m
Stagonomus amoenus (Brullé, 1832)	Yakamanastırı, 30.6.1998	Forest (Juniper, Quercus, Abies)	1270 m
Reduvidae			
Himacerus sp.	Kurucuova, 28.8.1998	Forest (Pinus)	1500 m
Rhinocoris iracundus (Poda, 1761)	Kurucaova, 30.6.1998	Forest (Pinus)	1500 m

Rhinocoris sp.	Martı Motel, 11.6.1998	Land and water lanes	1130 m
Rhophalidae			
Brachycarenus tigrinus (Schilling, 1817)	Dolama Ridge, 29.5.1998	Forest (Juniper)	1130 m
Chorosoma sp.	Near Çiftlikköy, 29.8.1998.	Agricultural area	1130 m
Corizus hyoscyami (Linnaeus, 1758).	Near Tolca, 4.7.1998	Land and water lanes	1130 m
Rhophalus sp.	Near Çiftlikköy, 29.5.1998	Agricultural area	1130 m
Scutelleridae			
Ancyrosoma leucogrammes (Gmelin, 1789)	Near Çiftlikköy, 29.8.1998	Agricultural area	1130 m
Eurygaster integriceps Put.	Near Tolca, 4.7.1998	Land and water lanes	1130 m
Eurygaster maura (Linnaeus, 1758)	Dolama Ridge, 29.5.1998	Forest (Juniper)	1130 m
Derula flavoguttata Mulsant et Rey, 1856	Dolama Ridge, 29.5.1998	Forest (Juniper)	1130 m
Odontotarsus sp.	Dolama Ridge, 29.5.1998	Forest (Juniper)	1130 m
Stenocephalidae			
Dicranocephalus albipes (Fabricius,1781)	Yakamanastırı, 30.8.1998	Forest (Juniper, Quercus, Abies)	1270 m
Tingidae			
Catoplatus sp.	Kurucuova, 30.6.1998	Forest (Pinus)	1500 m
Dictyla echii (Schrank, 1782)	Dolama Ridge, 29.5.1998	Forest (Juniper)	1130 m
Stephanitis sp.	Dolama Ridge, 30.6.1998	Forest (Juniper)	1130 m
Tingis sp.	Dolama Ridge, 29.5.1998	Forest (Juniper)	1130 m
HOMOPTERA			
Aphididae			
Aphis sp.	Dolama Ridge, 29.5.1998	Forest (Juniper)	1130 m
Myzus persica (Sulzer)	Martı Motel, 1.7.1998	Land and water lanes	1130 m

Cercopidae			
Cercopis vulnerata Rossi, 1807	Dolama ridge, 29.5.1998; Near Çiftlikköy, 11.6.1998;	Forest (Juniper); Agricultural area	1130 m, 1130 m
Cercopis sp.	Near Çiftlikköy, 28.5.1998	Agricultural area	1130 m
Cicadellidae			
Eupelix sp.	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Macrosteles sp.	Eskikanal 1st Bridge (Bekdemir), 11.6.1998	Agricultural area	1135 m
Cixiidae			
Pentastiridius sp.	Kurucuova, 1.7.1998	Forest (Pinus)	1500 m
Dictyopharidae			
Dictyophara asiatica Melichar,	Yakamanastırı, 29.8.1998	Forest (Juniper, Quercus, Abies)	1270 m
NEUROPTERA			
Ascalophidae			
Libelloides longicornis (Linnaeus, 1764)	Dolama ridge, 28.5.1999	Forest (Juniper)	1130 m
Chrysopidae			
Chrysoperla carnea (Stephens,)	Kurucaova, 1.7.1098; Yeşildağ, 1.7.1998.	Forest (Pinus, Quercus)	1500 m 1290 m
Myrmeleonidae			
Palpares libelluloides (Linnaeus, 1764)	3 km away from Yeşildağ, 30.6.1998.	Forest (Quercus)	1330 m
COLEOPTERA			
Adephaga			
Carabidae			
Carasus sp.	Near Çiftlikköy, 29.5.1998, 11.6.1998	Agricultural area	1130 m
Lebia cyanocephalus (Linnaeus, 1758)	Çiftlikköy locality, 29.5.1998	Agricultural area	1130 m

Zabrus sp.	Kurucaova, 28.5.1999, Martı Motel –Beyşehir, 2.7.1998.	Forest (Pinus), Land and water lanes	1500 m 1125 m
Cicindellidae			
Cicindella sp.	Kurucaova, 28.5.1999	Forest (Pinus)	1500 m
Polyphaga			
Anthicidae			
Anthicus sp.	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Anthribidae			
Bruchela suturalis (Fabricius, 1792)	Eskikanal (Bekdemir), 11.6.1998	Agricultural area	1135 m
Bruchela sp.	Eskikanal(Bekdemir), 11.6.1998	Agricultural area	1135 m
Apionidae			
Apion miniatum Germar;	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Attelabidae			
Rhynchites hungaricus Herbst.	Damla district. 31.5.1998	Forest (Quercus, Juniper, Berberis)	1155 m
Bruchidae			
Bruchidius canus (Germar, 1824)	Bademli, 12.6.1998	Steppe	1140 m.
Bruchidius foveolatus (Gyllenhal, 1833)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Bruchidius holosericeus (Schönherr, 1832)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Bruchidius poecilus (Germar, 1824)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Bruchidius pusillus (Germar, 1824)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Bruchidius sericatus (Germar, 1824)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Bruchidius tibialis (Boheman, 1829)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Bruchidius varius (Olivier, 1795)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m

Bruchus brachialis Fahraeus, 1839	Eskikanal 1st Bridge (Bekdemir), 11.6.1998; Kubad-Abad, 2.7.1998	Agricultural area	1125 m, 1130 m
Bruchus emerginatus Allard, 1868	Kubad-Abad, 2.7.1998	Agricultural area	1130 m
Bruchus lentis Frölich, 1799	Eskikanal 1st Bridge (Bekdemir), 11.6.1998	Agricultural area	1135 m
Bruchus rufipes Herbst, 1783	Eskikanal 1st Bridge (Bekdemir), 11.6.1998	Agricultural area	1135 m
Bruchus tristiculus Fahraeus, 1839	Kubad-Abad, 2.7.1998	Agricultural area	1130 m
Bruchus viciae Olivier, 1795	Üstünler way, 29.5.1998	Forest (Pinus)	1250 m
Spermaphagus calystegiae (Luk. And. T.-M., 1833)	Eskikanal 1st Bridge (Bekdemir), 11.6.1998	Agricultural area	1135 m
Spermaphagus kuesteri Schilsky, 1905	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Spermaphagus sericeus (Geoffroy, 1785)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Buprestidae			
Agrillus sp.	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Anthaxia hungarica (Scopoli, 1772)	Karaburun way, 31.5.1998	Forest (Quercus)	1130 m
Anthaxia sp.	Çiftlikköy locality, 11.6.1998	Agricultural area	1130 m
Cantharidae			
Cantharis bilunata Marsham, 1822	Near Akburun, 11.6.1998	Agricultural area	1170 m
Cerambycidae			
Acanthocinus griceus Fabricius,	Kurucaova, 28.5.1999	Forest (Pinus)	1500 m
Agapanthia lateralis Ganglbauer, 1884	Near Çiftlikköy, 3.7.1998	Agricultural area	1130 m
Agapanthia kirby (Gyllenhal, 1817)	Karaburun, 31.5.1998	Forest (Quercus), water lanes	1130 m

Agapanthia villosoviridescens (Degeer, 1775)	Near Çiftlikköy, 11.6.1998	Agricultural area	1130 m
Leptura sp.	Near Çiftlikköy, 28.5.1999	Agricultural area	1130 m
Phytoecia coerulescens (Scopoli, 1763)	Near Akburun, 11.6.1998	Agricultural area	1170 m
Purpuricenus budensis (Götz, 1783)	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Oberea oculata (Linnaeus, 1758)	İlırmak River-Yeşildağ, 2.7.1998	Agricultural area, water lanes	1125 m
Chrysomelidae			
Chrysolina amasiensis (Weise, 1894)	Şamlar Road-Wheat field, 30.5.1998	Steppe	1130 m
Clytra valerianae Menetries, 1832	Eskikanal (Bekdemir), 11.6.1998	Agricultural area	1150 m
Cryptocephalus bipunctatus (Linnaeus, 1758)	Near Üstünler Road, 29. 5.1998	Forest (Quercus, Juniper)	1200 m
Cryptocephalus concolor Suffrian, 1847	Eskikanal (Bekdemir), 11.6.1998	Agricultural area	1135 m
Cryptocephalus connexus Olivier, 1808	Kurucaova, 30.6.1998	Forest (Pinus)	1500 m
Entomoscelis adonidis (Pall.,1771)	Near Çiftlikköy, 11.6.1998	Agricultural area	1130 m
Gastrophysa polygoni (Linneaus,1758)	Eskikanal (Bekdemir), 11.6.1998	Agricultural area	1135 m
Smaragdina limbata (Steven, 1806)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Hippocassida supferruginae (Schr.,1776)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Labidostomis longimana (Linnaeus, 1758)	Karaburun-Gavur Reed Bed, 31.5. 1998	Land and water lanes	1130 m
Luperus longicornis (Fabricius, 1781)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Leptinotarsa decemlineata (Say, 1824)	Near Laleli Month, 28.5.1999	Agricultural area	1150 m

Chrysomela populi Linneaus, 1758	Bekdemire 1 km. kala,11.6.1998	Agricultural area	1170 m
Pachybrachys fimbriolatus Weise,1882	Karaburun-Gavur Reed Bed, 31.5. 1998	Land and water lanes	1130 m
Pachybrachys limbatus (Lopatin, 1992)	Karaburun-Gavur Reed Bed, 31.5. 1998	Land and water lanes	1130 m
Pachybrachys misellus (Weise, 1900)	Şamlar Road-Wheat field, 20.5.1998	Step	1130 m
Cleridae			
Trichodes quadriguttatus Adams, 1817	Near Çiftlikköy, 11.6.1998	Agricultural area	1130 m
Coccinellidae			
Adalia bipunctata (Linnaeus, 1758).	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Adonia variegata (Goeze, 1777)	Eskikanal (Bekdemir), 11.6.1998	Agricultural area	1135 m
Coccinella semptempuctata (Linneaus, 1758)	İliırmak River, 3.7.1998	Agricultural area	1125 m
Scymnus apetzi Mulsant, 1846	Eski kanal (Bekdemir), 11.6.1998; Çiftlikköy, 4.7.1998	Agricultural area, Agricultural area	1135 m 1135 m
Thea 22-punctata (Fabr.)	Çiftlikköy, 4.7.1998	Agricultural area	1130 m
Curculionidae			
Bangasternus orientalis Capiomont, 1873	Şamlar Road, 20.5.1998	Steppe	1130 m
Cionus sp.	Dolama Ridge, 30.6.1998	Forest (Juniper)	1130 m
Curculio sp.	Yakamanastırı, 29.5.1998	Forest (Juniper, Quercus, Abies)	1270 m
Gymnetron asellus (Gravenhorst, 1807)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Gymnetron tetrum (Fabricius,1792)	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Gymnetron sp.	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m

Hylobius abietis Linneaus, 1758	Kurucaova, 28.5.1999	Forest (Pinus)	1470 m
Hypera sp.	Yakamanastırı, 29.5.1998	Forest (Juniper, Quercus, Abies)	1270 m
Larinus jacaea Fabricius,1775	Çiftlikköy locality, 28.5.1999	Agricultural area	1130 m
Larinus latus (Herbst, 1784)	Çiftlikköy locality, 11.6.1998	Agricultural area	1130 m
Larinus sp.	Çiftlikköy locality, 11.6.1998	Agricultural area	1130 m
Lixus cardui Olivier,1807	Çiftlikköy locality, 11.6.1998	Agricultural area	1130 m
Lixus elongatus Gocze, 1777	Dolama Ridge, 28.5.1999	Forest (Juniper)	1130 m
Magdalis frontalis (Gyllenhal, 1827)	Yakamanastırı, 29.5.1998	Forest (Juniper, Quercus, Abies)	1270 m
Mogulones lineatus (Gyllenhal, 1837)	Near Çiftlikköy, 11.6.1998	Agricultural area	1130 m
Otiorhynchus anatolicus Boheman, 1843	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Polydrusus gracillicornis Kiesenwetter, 1864	Yakamanastırı, 30.6.1998	Forest (Juniper, Quercus, Abies)	1270 m
Polydrusus ponticus Faust, 1888	Eskikanal Bekdemir, 11.6.1998	Agricultural area	1135 m
Polydrusus picus (Fabricius, 1792)	Sarpça Tepe, 31.5.1998	Steppe	1125 m
Sibinia syriaca Faust, 1890	Yakamanastırı, 29.5.1998	Forest (Juniper, Quercus, Abies)	1270 m
Hydrophilidae			
Hydrous piceus Linneaus,	Martı Motel – Beyşehir, 30.5.1998	Land and water lanes	1125 m
Malachidae			
Malachius bipustulatus (Linnaeus, 1758)	Dolama ridge, 30.6.1998	Forest (Juniper)	1130 m
Meloidae			
Alosimus sp.	Near Akburun, 11.6.1998	Agricultural area	1170m

Lydus trimaculatus (Fabricius,1775)	3 km away from Yeşildağ Chrome Foundation vicinity, 30.6.1998	Agricultural area	1125 m
Lydus sp.	Near Çiftlikköy, 29.5.1998	Agricultural area	1130 m
Meloe sp.	Çiftlikköy, 17.10.1998	Agricultural area	1130 m
Mylabris cincta Olivier, 1811	Kurucaova, 28.8.1998, Kubad-Abad, 2.7.1998	Forest (Pinus); Agricultural area	1500 m; 1138 m
Mylabris crocata Pallas, 1781	Kubad-Abad, 2.7.1998	Agricultural area	1138 m
Mylabris polymorpha (Pallas, 1771)	3 km away from Yeşildağ Chrome Foundation vicinity, 30.6.1998	Agricultural area	1125 m
Mylabris sp.	Kurucaova, 30.6.1998	Forest (Pinus)	1500 m
Melyridae			
Enicopus pilosus (Scopoli, 1763)	Şamlar Road, 20.5.1998	Steppe	1130 m
Nitidulidae			
Corpophilus sp.	Çiftlikköy locality, 11.6.1998	Agricultural area	1130 m
Scarabaeoidae			
Aphodiidae			
Apodius sp.	Near Tolca, 4.7.1998	Land and water lanes	1130 m
Scarabaeidae			
Amphicoma sp.	Apsıngır, 29.5.1998	Forest (Juniper, Quercus)	1300 m
Anisoplia segetum (Herbst, 1783)	Çiftlikköy locality, 11.6.1998.	Agricultural area	1130 m
Anisoplia austriaca Herbst 1783	Çiftlikköy locality, 4.7.1998	Agricultural area	1130 m
Anoxia sp.	Çiftlikköy locality, 3.7.1998	Agricultural area	1130 m
Cetonia aeruginosa (Drury, 1770)	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Cetonia aurata (Linnaeus, 1761)	İğdeli Island, 2.7.1998	Land and water lanes	1130 m

Eulasia hibrida (Reitter, 1890)	1 km away from Bekdemir, 11.6.1998	Agricultural area	1170 m
Netocia hungarica (Herbst, 1790)	İğdeli Island, 2.7.1998	Land and water lanes	1130 m
Onthopagus gibbulus (Pallas, 1781)	Ortaköy, 2.7.1998	Agricultural area	1130 m
Oryctes nasicornis Linnaeus, 1758	Martı Motel – Beyşehir, 11.6.1998	Land and water lanes	1125 m
Oxythyrea cinctella (Schaum, 1841)	Dolama ridge, 30.6.1998	Forest (Juniper)	1130 m
Potasia cuprea (Andersch, 1797)	Dolama ridge, 30.6.1998	Forest (Juniper)	1130 m
Tropinota hirta Poda,1761	Şamlar Road, 30.6.1998	Agricultural area	1135 m
Tenebrionoidae			
Alleculidae			
Omophlus sp.	Dolama ridge, 30.6.1998	Forest (Juniper)	1130 m
Podonta sp.	Eskikanal 1st Bridge(Bekdemir), 11.6.1998	Agricultural area	1135 m
Tenebrionidae			
Blaps sp.	Çiftlikköy locality, 11.6.1998	Agricultural area	1130 m
Dailognatha sp.	Kubad-Abad, 2.7.1998	Agricultural area	1130 m
Prosodes sp.	Yakamanastırı, 28.5.1999	Forest (Juniper, Quercus, Abies)	1270 m
Tentyria herculeana Germar, 1824	Yakamanastırı, 30.6.1998	Forest (Juniper, Quercus, Abies)	1270 m
Staphylinidae			
Stenus sp.	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Tachyporus hypnorum (Fabricius, 1775)	Yakamanastırı, 29.5.1998	Forest (Juniper, Quercus, Abies)	1270 m
DİPTERA			
Asilidae			
Dasyopogon diedema (Fabricius, 1781)	Gölkaşı Island, 2.7.1998	Forest (Quercus)	1130 m

Machimus sp. 1	Ortaköy, 2.7.1998	Agricultural area	1130 m
Machimus sp. 2	Gölkaşı Island, 2.7.1998	Forest (Quercus)	1130 m
Machimus setibarbus (Loew, 1849)	Gölkaşı Island, 2.7.1998	Forest (Quercus)	1130 m
Promachus canus Walker, 1865	Çiftlikköy locality, 11.6.1998	Agricultural area	1130 m
Stenopogon sabaudus (Fabricius, 1794)	Çiftlikköy locality, 11.6.1998	Agricultural area	1130 m
Stenopogon sp.	Çiftlikköy locality, 11.6.1998	Agricultural area	1130 m
Bombyliidae			
Bombylius sp.	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Callyphoridae			
Lucilia sp.	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Culicidae			
Anopheles maculipennis Meigen, 1818	Martı Motel - Beyşehir, 3.7.1998	Land and water lanes	1125 m
Culex pipiens (Linnaeus, 1758)	Martı Motel - Beyşehir, 3.7.1998	Land and water lanes	1125 m
Opomyzidae			
Thaumatomyia sp.	Yakamanastırı, 30.6.1998	Forest (Juniper, Quercus, Abies)	1270 m
Syrphidae			
Eristalis sp.	Çiftlikköy locality, 11.6.1998	Agricultural area	1130 m
Tabanidae			
Chrysops flavipes Meigen, 1804	Near Tolca, 4.7.1998	Land and water lanes	1130 m
Dasyramphis umbrinus Meigen, 1820	Yakamanastırı, 29.5.1998	Forest (Juniper, Quercus, Abies)	1270 m
Pangonius pyritosus Loew, 1859	Çiftlikköy locality, 3.7.1998	Agricultural area	1130 m
Philipomyia graeca (Fabricius,1794)	Dolama ridge, 30.6.1998	Forest (Juniper)	1130 m
Tabanus bifarius Loew, 1858	Yeşildağ, İlırmak River, 3.7.1998	Agricultural area, water lanes	1125 m

Tabanus lunatus Fabricius, 1794	Yakamanastırı, 30.6.1998	Forest (Juniper, Quercus, Abies)	1270 m
Tabanus martinii Kröber, 1928	Yakamanastırı, 30.6.1998	Forest (Juniper, Quercus, Abies)	1270 m
Tabanus quatuornatus Meigen, 1820	Bayramköy-Beyşehir, 12.6.1998	Agricultural area , Forest (Pinus)	1250 m
Tabanus spodopterus Meigen,1820	Yakamanastırı, 30.6.1998	Forest (Juniper, Quercus, Abies)	1270 m
Therioplectus tricolor Zeller, 1842	Yakamanastırı, 30.6.1998	Forest (Juniper, Quercus, Abies)	1270 m
HYMENOPTERA			
Andrenidae			
Andrena pyropyga	Kubad-Abad, 2.7.1998	Agricultural area	1130 m
Apidae			
Bombus terrestris (Linnaeus,1758)	Şamlar Road, 30.6.1998	Agricultural area	1140 m
Megabombus zonatus (Smith,1854)	Çiftlikköy locality, 4.7.1998	Agricultural area	1130 m
Halictidae			
Halictus sp.	Near Tolca, 4.7.1998	Land and water lanes	1130 m
Ichneumonidae			
**Barycnemis* sp. New record	Bayındır, 12.6.1998	Steppe	1200 m
Cymodusa sp.	Eskikanal Bekdemir, 11.6.1998	Agricultural area	1135 m
Itoplectis maculator Fabricius 1775	İlıırmak River, 3.7.1998	Agricultural area	1125 m
Sinophorus sp.	Kurucaova, 3.7.1998	Forest (Pinus)	1500 m
Tryphon sp.	Eskikanal Bekdemir, 11.6.1998	Agricultural area	1135 m
Scoliidae			
Megascolia flavifrons Fabricius, 1787	Çiftlikköy locality, 3.7.1998	Agricultural area	1130 m
Scolia hirta ssp. *Unifasciata* Cyrillo,	Aygır Island, 2.7.1998	Land and water lanes	1130 m
TRICHOPTERA			
Ecnomidae			
Ecnomus tenellus Rambur, 1842	Martı Motel – Beyşehir, 3.7.1998	Land and water lanes	1125 m

Glossosomatidae			
Synagapetus anatolicus Cakin, 1983	Kurucaova, 21.7.1998	Forest (Pinus)	1450 m
Hydropsychidae			
Hydropsyche kebab Malicky, 1974	Kurucaova, 21.7.1998	Forest (Pinus)	1350 m
Hydropsyche bulbifera McLachan, 1878	Kurucaova, 21.7.1998	Forest (Pinus)	1300 m
Hydroptilidae			
Allotrichia pallicornis (Eaton,1873).	Yeşildağ, İlıırmak River, 22.7.1999	Agricultural area, water lanes.	1125 m
Oxyethira falcate Morton, 1893	Martı Motel - Beyşehir, 18.7.1998	Land and water lanes	1125 m
Leptoceridae			
Athripsodes longispinosus Martynov, 1909	Martı Motel – Beyşehir, 18.7.1998; Yeşildağ, İlıırmak River, 22.7.1999	Land and water lanes; Agricultural area, water lanes	1125 m
Leptocerus interruptus Fabricius, 1775	Yeşildağ, İlıırmak River, 22.7.1999	Agricultural area, water lanes	1125 m
Leptocerus tineiformis Curtis, 1834	Yeşildağ, İlıırmak River, 22.7.1999	Agricultural area, water lanes	1125 m
Oecetis ochracea Curtis, 1825	Martı Motel – Beyşehir, 3.7.1998	Land and water lanes	1125 m
Limnephilidae			
Drusus gueneri Sipahiler, 1995	Kurucaova, 21.7.1998	Forest (Pinus)	1450 m
Grammotaulius nigropunctatus Retzius, 1793	Yakamanastırı, 29.5.1998	Forest (Juniper, Quercus, Abies)	1270 m
Limnephilus flavospinosus Stein, 1874	Dolama ridge, 29.5.1998	Forest (Juniper)	1130 m
Limnephilus sp.	Yakamanastırı, 28.5.1999	Forest (Juniper, Quercus, Abies)	1270 m
Psychomyiidae			
Tinodes pluvialis Malicky, 1987	Kurucaova, 21.7.1998	Forest (Pinus)	1350 m
Phryganeidae			
Phryganea grandis serti ssp. n. Sipahiler, 2000	Gedikli, Lake lanes, 11.6.1998	Land and water lanes	1130m

Sericostomatidae			
Sericostoma flavicorne Schneider, 1845	Kurucaova, 21.7.1998	Forest (Pinus)	1350 m
LEPİDOPTERA			
Nymphalidae			
Cynthia cardui (Linnaeus, 1758)	Gölkaşı Island, 2.7.1998;	Forest (Quercus)	1130 m
Issoria lathonia (Linnaeus, 1758)	Yakamanastırı, 30.6.1998	Forest (Quercus, Juniper, Abies)	1400 m
Pieridae			
Artogeia sp.	Kubad-Abad, 2.7.1998	Agricultural area	1130 m
Gonepteryx rhamni (Linnaeus,1758)	Beyşehir, 1.7.1998	Forest (Quercus), Agricultural area	1130 m
Satyridae			
Brintesia circe (Fabricius, 1775)	Dolama ridge, 30.8.1998	Forest (Juniper)	1130 m
Melanargia Larissa (Geyer, 1828)	Dolama ridge, 30.8.1998	Forest (Juniper)	1130 m
Zygaenidae			
Zygaena purpuralis (Brünnich,1763)	Kubad-Abad, 2.7.1998	Agricultural area	1130 m

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SALT STRESS-INDUCED CHANGES IN FREE AND BOUND POLYAMINE LEVELS IN SUNFLOWER (*Helianthus annuus* L.) SEEDS DIFFERING IN SALT TOLERANCE[1]

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Abstract

In the present study, the effect of salt stress (50, 100, 150mM NaCl) on the endogenous levels of free, bound and total polyamines was studied in dry (Control) and imbibed seeds of salt tolerant (Coban) and salt sensitive (Sanbro) cultivars of sunflower (*Helianthus annuus* L.) plants. Levels of **free Spm, Spd, AS-bound Spd** and **total polyamine** were found to be higher in dry seeds of salt sensitive Sanbro than salt tolerant Coban cultivar. Imbibing the seeds, for 24 hours, in different concentrations of saline culture solutions, caused to decrease the **free** and **AS-bound Spm** levels only in Sanbro but decreased **free, AS-bound, AINS-bound** and **total Spd** with **AINS-bound** and **total Spm** levels in both cultivars. The **free Put** could not be detected in dry seeds whereas a trace amount of Put was found in the imbibed seeds.

Keywords: sunflower, salinity, polyamines (free, bound and total).

Abbreviations: AINS-bound, acid insoluble-bound; AS-bound, acid soluble-bound; Put, putrescine; Spd, spermidine; Spm, spermine

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Introduction

Environmental stress is the major factor limiting plant productivity (1). Abiotic stresses like drought, temperature and salinity, which cause depletion of cellular water, are responsible for the greatest agricultural losses. Upon exposure to these prevalent stresses, many plants accumulate organic osmolytes, most commonly polyhydroxylic compounds (saccharides and polyhydric alcohols) and zwitterionic alkylamines (aminoacids and polyamines). Polyamines (PAs) like putrescine (Put), spermidine (Spd) and spermine (Spm) are polycationic compounds present in all living organisms. PAs and their biosynthetic enzymes have been implicated in a wide range of metabolic processes in plants, ranging from cell division and organogenesis to the protection against stress (2, 3, 4). In many plant systems, polyamines do not only occur as free molecular bases, but can also be covalently linked to monomers of phenolic acids, mostly hydroxycinnamic acids (acid-soluble conjugated polyamines) as well as to dimers and trimers of phenolic acids or other high molecular-mass substances like proteins via transglutaminases (acid insoluble conjugated polyamines).

PA levels may be an integral part of the response mechanism of plants to various stresses, such as mineral nutrient deficiencies, temperature stress, hypoxia, low external pH, osmotic stress and salinity (5, 6, 7, 8, 9, 10, 11).

Differences in PA response under salinity stress have been reported among and within plant species. According to Prakash et al. (12) endogenous levels of PAs decreased in rice seedlings under NaCl stress, whereas Basu et al. (13) reported that salinity results in accumulation of PAs in the same material. Since metabolism of polyamines under saline situations is not properly understood, the role of polyamines in relation to salt tolerance still remains obscure (14). Moreover specific information on the effects of salinity on PA levels in sunflower is very scarce. In order to understand salinity induced changes in the titers of polyamines and to achieve a possible correlation between the levels of polyamines and degree of salt tolerance in sunflower the present study, using salt sensitive and salt tolerant sunflower cultivars was undertaken with the objectives to evaluate the metabolic levels of free and bound (acid soluble and acid insoluble) polyamines, in dry and soaking seeds under increasing levels of NaCl salinity.

Materials and Methods

Plant material

In this study, seeds of sunflower (*Helianthus annuus* L.) cultivars like Coban (salt tolerant) and Sanbro (salt sensitive) were used. The seeds were obtained from Seed Improvement and Certification Center in Turkey. From the percent of germination studies, cultivar Sanbro was found to be salt sensitive and cultivar Coban was salt tolerant.

Selected seeds of uniform in size were soaked and imbibed with different concentration of saline culture solutions for overnight. The culture solutions used were half-strength Hoagland solution (15) and Hoagland-salinized with NaCl at concentrations of 50, 100 and 150mM. The imbibed seed material were quickly frozen with liquid nitrogen and stored at –20 °C for analyses.

Determination of Polyamines

Free PA determination was done by method of Flores and Galston (16) with some modifications. Bound PA's were determined in acid hydrolyzed supernatants (acid soluble-bound polyamines) and acid hydrolyzed pellets (acid-insoluble polyamines) according to Tiburcio et al. (17). 200 mg weighed dry or imbibed seed material was homogenized in 2 ml 5% $HClO_4$ in a chilled mortar. The homogenates were placed in ice for about 30 min and then centrifuged at 4 °C at 15.000 rpm for 20 min. The pellets, after washing twice in two volumes of perchloric acid (PCA), were resuspended in the original volume of PCA. Replicates (0.2 ml) of this suspension and original supernatant were put in glass ampoules together with the same volume of 12 M HCl. The ampoules were flame sealed and incubated at 110 °C for 20 h to hydrolyze their content. The hydrolysates were dried in a water bath at 60 °C and resuspended in th original volume of PCA. Aliquots (0.2 ml) of the PCA supernatant (free polyamines), of the same hydrolyzed supernatant (acid soluble- bound polyamines; AS-bound) and of the suspension of the hydrolyzed pellet (acid insoluble-bound polyamines; AINS-bound) were dansylated and separated on high performance thin layer chromatography (HPTLC) plates (Whatman LK 6D) with concentrating zone, using cyclohexane: ethylacetate (3:2, v/v) as developing solvent.

After chromatographic separation, free and bound polyamines were detected under UV light and quantified by densitometry (Pelkin Elmer) with excitation and emission at 365 and 500 nm, respectively. Polyamines were quantified by comparison of the integration areas from the samples with those of pure standards, treated in the same way.

Statistical Analysis

The experiments were performed in a factorial design with three replicates. Analysis of variance of the results was performed and compared with least-significant differences (LSD) at the 5% level.

Results and Discussion

Effect of salinity on PAs (free, bound and total) level

In dry seeds **free Spm, Spd** and **AS-bound Spd** and **total polyamine** levels are found to be higher in Sanbro than Coban ($P<0.05$). Furthermore, **total polyamine** levels in Sanbro seeds are twice more than in Coban seeds (Table 1).

The **free PA** levels in Sanbro and Coban seeds soaked with saline culture solutions at different concentrations show significant decreases in comparison to the control groups soaked with Hoag. solution ($P<0.05$) (Table 2).

Soaking the Sanbro seeds either in Hoag. or one of the saline cultures caused to decrease the **free Spm and Spd** levels but soaking the Coban seeds appears to have increased levels of **free Spm** and decreased levels of **free Spd** in comparison to the dry seeds (Table 1 and 2). Anguillesi et al. (18) obtained the similar findings with *Glycine max*, *Helianthus annuus* and *Triticum* seeds.

Soaking Sanbro seeds, for 24 hours, in some different concentrations of saline culture solutions, also caused to decrease **AS-bound Spm** level but in both cultivars **AS-bound, AINS-bound** and **total Spd** with **AINS-bound** and **total Spm** levels were decreased in comparison to the control (Hoag.) groups ($P<0.05$). The **free Put** could not be detected in dry seeds whereas a trace amount of Put was found in the soaked seeds. These findings are similar to those of previous studies (12, 19). Furthermore Torrigiani et al. (20) found that Spd and Spm levels decreased during soaking the seeds of *Zea mays* but the Put amount increased.

In both imbibed and dry seeds, in both cultivars **free Spd** was found at the highest rate. The rates of others were identified as follows: **AS-bound Spd** and **free Spm.** This finding is supported by Angosto and Matilla (21) and Lin et al (22). They found that free Spd was at the highest level among the other polyamine titers in the seeds of *Festuca* and *Glycine max* (L.) respectively.

Total bound polyamine (**AS-bound** and **AINS-bound** polyamine) level forms the great part of total free polyamine level respectively 43.4% in Sanbro and 56.05% in Coban

Table 1. Free and bound (acid soluble and acid insoluble) polyamine contents (nmol/ g) in dry seeds of *Helianthus annuus* L. cultivars. nd:not detected, (mean±SE)

		POLYAMINE TITERS (nmol /g)				
CULTIVARS	**PAs**	**FREE**	**AS-BOUND**	**AINS-BOUND**	**TOTAL (free+bound)**	**TOTAL**
SANBRO	**Spm**	39.9±3.4	15.4±1.5	3.1±0.5	58.4	312.5
	Spd	212.1±3.9	42.0±4.9	eser	254.1	
	Put	nd	trace	trace	trace	
COBAN	**Spm**	14.4±1.7	9.5±1.3	2.8±0.7	26,7	155.7
	Spd	108.3±5.5	20.7±0.9	trace	129.0	
	Put	nd	trace	trace	trace	

Table 2. Effect of increasing NaCl concentrations on free, AS-bound and AINS-bound polyamine content (nmol/g) in soaking seeds of *Helianthus annuus* L. cultivars. nd:not detected, (mean±SE)

		POLYAMINE TITERS (nmol/g)												
CULTIVARS	TREATMENT	FREE			AS-BOUND			AINS-BOUND			TOTAL			
	(mM NaCl)	Spm	Spd	Put	Spm	Spd	Put	Spm	Spd	Put	Spm	Spd	Put	TOTAL
SANBRO	Hoag.	35.2±3.2	156.6±12.1	nd	25.3±2.7	37.9±1.3	nd	8.1±1.4	12.0±2.9	nd	68.6	206.45	_	275.05
	50	29.3±2.9	134.6±10.7	trace	21.4±3.1	31.7±4.2	trace	6.9±0.4	11.1±0.5	trace	57.57	177.35	_	234.92
	100	17.2±1.3	62.8±15.2	trace	15.4±1.7	22.6±1.6	trace	5.0±0.8	7.1±0.6	trace	37.65	92.55	_	130.2
	150	22.5±2.4	118.4±8.9	trace	18.1±2.1	15.2±0.7	trace	5.4±0.2	8.1±0.8	trace	46.02	141.71	_	187.73
COBAN	Hoag.	25.2±1.5	86.5±2.7	nd	15.4±2.9	42.0±4.9	nd	7.1±0.9	9.3±1.2	nd	47.65	137.77	_	185.42
	50	16.9±1.3	43.9±2.3	trace	14.1±2.3	37.9±1.3	trace	8.1±0.8	11.2±0.9	trace	39.09	92.96	_	132.05
	100	20.8±1.7	59.9±2.7	trace	11.8±1.3	20.1±0.5	trace	6.5±0.5	7.9±0.5	trace	39.10	87.86	_	126.96
	150	21.2±1.6	63.8±7.5	trace	11.6±0.4	13.2±0.2	trace	4.1±0.3	5.9±0.4	trace	36.9	82.89	_	119.79

seeds soaked with Hoag. solution (Table 2). This finding, as Bonneau et al. (23) argue, suggests that amines are closely related to seed germination and that bound amines may function as storage in the formation of free polyamine.

These findings indicate that polyamines are synthesized in both dry and soaking sunflower seeds and the synthesis decreases as a result of salt application. Those findings regarding polyamine levels in both dry and soaking seeds appear to be different from the other findings. These differences may result from seed type, seed volume, etc. The findings also suggest that the current information cannot provide a full explanation on the effects of polyamines under saline conditions on the development of seeds. However based on the findings it is possible to suggest that salt treatment may limit Put and other amine synthesis and therefore, it can inhibit the development of plant. Strogonov et al. (24) showed that salinization increased Put accumulation in Vicia *faba*, but not in barley and sunflower. The salt stress-induced modifications in PA concentrations reported in the present study concern only short-term exposure so that the long-term effects of salt stress may be quite differently achieved.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF THE VENOM FROM THE SCORPION *Mesobuthus gibbosus* (BUTHIDAE)

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Abstract

In this study, the crude venom, isolated from the scorpion *Mesobuthus gibbosus*, was analyzed for determining the neurotoxic peptides. Venom sample was injected into a RP-C4 Analytical HPLC column and fractions were collected manually into tubes. The crude venom was initially separated into 10 fractions. Each purification step was followed by bioassays and SDS-PAGE applications. Fraction M5 was lethal to mice. M5 was separated using the same system equipped with a RP-C18 column and obtained 3 fractions. Then collected manually into tubes by following at 214 nm. It was found that fraction M5 consisted of three peptides. The molecular weight of the neurotoxic fragment was 6600 Da.

Keywords : *Mesobuthus gibbosus*, HPLC purification, neurotoxic peptides

Introduction

Scorpions are among the oldest creatures on earth. The largest family, Buthidae, contains over 700 species. *Mesobuthus gibbosus* is belong to this family[1].

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Scorpion species of Turkey and their distribution are determined[2]. According to this documentation, this species' habitat is spread over the west side of Anatolian diagonal and all over the Anatolia except of Black Sea shore lane. Members of the family Buthidae are medically relevant due to their ability to produce potentially fatal secretions that are neurotoxic to humans[3]. Scorpion venom is a complex, water-soluble, antigenic poison composed of mucopolysaccharides, hyaluronidase, phospholipase, serotonin, histamine, protease inhibitors, histamine releasers, and neurotoxins [4,5].

Scorpion venom is a rich source of polypeptide neurotoxins that affect ion channels specifically; they can be classified into two groups according to their molecular sizes, namely long-chain and short-chain neurotoxins. Among the long chain neurotoxins some are only toxic to mammals[6] and others are insect-specific neurotoxins[7,8]. A large number of scorpion neurotoxins are especially able to modify the normal function of sodium channels[9]. They have a high affinity for a specific binding site on the sodium channel of excitable membranes and they induce a prolongation of the axonal action potential by slowing down the inactivation process of the channel. Although the toxic components vary both immunologically and pharmacologically, symptomatology in envenomated humans is quite uniform[10]. Scorpion envenomation is an important health problem in some regions in the world . Venoms contain toxins that are likely to be responsible for the noxious effects observed when people are stung by scorpions. Neutralization of these molecules is an important challenge and the use of monoclonal antibodies (mAbs) may be advantageous. Recent advances in antibody engineering methodology have enhanced the field of mAb applications. *M. gibbosus* is the main species of West Anatolia and a serious threat for humans, especially in the summer.

In this study the neurotoxic components of *M. gibbosus* obtained from Manisa were investigated by HPLC and fractions were analyzed for their biological activity.

Materials and Methods

a) Source of Venom

Scorpion venom was obtained from captive scorpions by electric stimulation (rectengular pulses of 25 V, 1 msecond duration at a frequency of 2,5 Hz using a CFP stimulator Model 8048) of the posterior abdomen of scorpions. The scorpion venom used was extracted from *M. gibbosus* scorpions collected in rural areas of Manisa, Turkey. Total venom was extracted by electrical stimulation and lyofilised for further analysis.

b) Sample Preparation

The venom was re-suspended in bidistilled water and centrifuged at 15,000 g for 20 min. The supernatant containing the soluble fraction of venom was aliquoted, freeze-dried and kept at -20 °C until use. The aliquots needed for each application were thawed and used.

c) Chemicals

The solvents used were HPLC grade and water was bidistilled.

d) HPLC Purification

Venom sample was injected into a Vydac (Hesperia, CA, USA) RP-C4 Analytical HPLC column (4.6 mm internal diameter x 250 mm length) connected to Shimadzu LC-10 ADVP, SPD-10 AVVP detector. A gradient was formed with the following conditions: 5-65% solvent A in 60 min, 65% solvent A for another 20 min for a total of 80 min at a flow rate of 600 µL per minute (column pressure: 980 psi) (solvent A, 95% acetonitrile, 5% water, 0.1 % trifluoroacetic acid). Elution was monitored by following the UV trace at 214 and 280 nm. Fractions were collected manually into tubes. Biologically active fractions were further separated using the same system equipped with a RP-C18 column (4.6 mm internal diameter x 250 mm length) and an online 5 µ peptide trap (Nucleosil 100). With a linear gradient from 0 to 50% solvent A in 60 min at flow rate of 1 mL/min (column pressure: 1300 psi). Fractions were collected manually into tubes by following the UV trace. The UV detection was performed at 214 nm and 280 nm. The chromatographic analyses and columns were run at room temperature (25 °C).

e) Bioassays

Mice were purchased from Hacettepe University Animal Housing Facility. Purified and collected fractions were tested for *in vivo* toxicity on 20±2 g C57/BL6 mice. Mice were anaesthetized with diethylether, injected in the left cerebral ventricle with 1µL of sample was dissolved in a solution of bovine serum albumin (BSA) (0.25 mg/mL in 0.9 % NaCl) and placed in glass jars for observation. Control animals injected with BSA did not show any symptoms when recovering from anesthesia. The following signs of toxicity were assessed: excitability, salivation, trembling of the legs and body, jerking of the limbs, loss of ability to walk and death. Appearance of symptoms was noted continuously during the first hour of injection and was monitored at regular intervals for 24 h or until death.

These fractions were also tested on *Musca domestica* larvae, which were obtained from Hacettepe University Department of Biology, Ecology Section. Five larvae were used per fraction. A volume of 1µL was injected into the abdominal segment. Observation of contractions and/or paralysis and death was made at 5 min, 15 min, 60 min and 24 h post-injection.

a) Molecular Weight Determination of Fractions

Molecular weight of the crude venom and chromatographic fractions were determined according to the method of Laemmli[11]. Polyacrilamide gel electrophoresis (PAGE) was used to estimate molecular weight and also as a criterion of purity.

PAGE was performed using the following protocol: resolving gel was prepared as 15% in 1.5 M Tris-HCl buffer, pH 8.8. Acrylamide/bisacrilamide (30% T, monomer concentration) were polymerized by 10% APS (ammonium persulphate) and TEMED (N,N,N',N' - tetramethylethylenediamine) for 45 minutes. A 3.9 % stacking gel (in Tris-HCl buffer, pH 6.8) was applied above the resolving gel. For SDS-PAGE applications, resolving gel was prepared as 15% in 1.5 M Tris-HCl buffer with 10%SDS (sodium dodecyl sulphate), pH 8.8. In each run 10 µL of sample was taken from the lyofilised stocks and diluted as 1:1 with the sample buffer consisted of 0.5 M Tris, pH 6.8, glycerol, and 0.05% bromophenol blue. Diluted samples were heated at 95^{0}C for 4 minutes. 10 µL of diluted samples and low range protein molecular weight standard marker was (Sigma Marker™, Product No. M 3788) then loaded into the wells with a Hamilton syringe at about 1-2 mm far from the well bottom. Electrophoresis was performed at a current voltage of 200 V for 45 minutes. After electrophoresis, gels were stained in 0.1.% Coomasie Blue R-250 prepared in 40% methanol and 10% acetic acid for one hour. Gels were destained in 10% acetic acid and 40% methanol for 1-3 hours to visualize the protein bands. Molecular weights of the samples were determined by the aid of the calibration curve obtained by plotting log molecular weight versus relative mobility for a group of standard proteins since the molecular weight is along function of relative mobility. The relative mobility of the samples was then fitted to the curve to determine their molecular weights.

Results

In this study source of venom was adult *Mesobuthus gibbosus* scorpions (Figure 1). Neurotoxins in the crude venom from this species were analyzed by HPLC. For molecular weight determination, we have utilized SDS-PAGE to separate different

components of the fractions. Optimization of the HPLC gradient conditions resulted in separation of the venom into 10 fractions (Figure 2).

Figure 1. *Mesobuthus gibbosus*

As seen in Figure 3, crude venom consisted of peptides with the molecular weights range between 6300-116000 Da. Soluble crude venom and each of the fractions were tested for activity against mice and *Musca domestica* larvae (Table 1). Fractions M4 and M8 had activity against larvae and fractions M4, M5, M8 had activity against mice. Fraction M4 was specific for larvae because of moderately high contractive effect. Fraction M5 was very effective against mice.

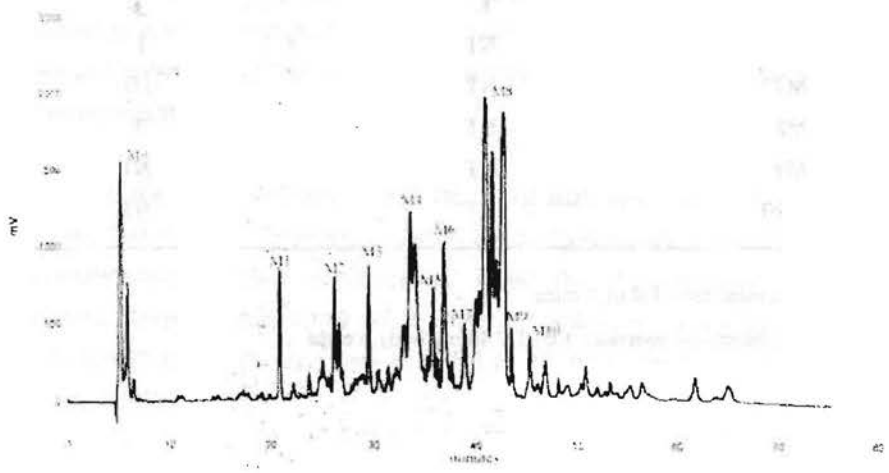

Figure 2. Separation of crude venom by RP-HPLC using a C4 column. The separation was established as explained in Materials and Methods section. Individual peaks (M0, M1, M2, M3, M4, M5, M6, M7, M8, M9 and M10) were collected, lyofilized and used for molecular weight analysis and bioassays.

Fraction M5 (Figure 2) from the C4 column gave severe symptoms when injected to mice. The toxicity symptoms were intense with salivation and trembling and immediately death. Controls injected with BSA in saline rapidly recovered within several minutes.

This fraction (M5) was collected and for further purification applied to C18 column (Figure 4). It was found that fraction M5 consisted of three peptides with the molecular weights of 6300, 6400 and 6600 Da, respectively (Figure 5). After C18 purification, these fractions was collected and injected to mice and biological activity was confirmed (Table 2).

Table 1. Toxicological profile of the C4 fractions of the venom of *Mesobuthus gibbosus* [Toxic (T): Injected animals all showing signs of poisoning but symptoms; Lethal (L): Injected animals dead; NT (Non-toxic)]

Fraction	Mice [a]	*M. domestica* larvae [b]
M0	NT	NT
M1	NT	NT
M2	NT	NT
M3	NT	NT
M4	NT	NT
M5	**L**	**T**
M6	NT	L
M7	NT	NT
M8	NT	T
M9	NT	NT
M10	NT	NT

[a] i.c.v. injection ; 1.0 µL / mice

[b] postabdominal injection; 1.0 µL / 30 mg body weight

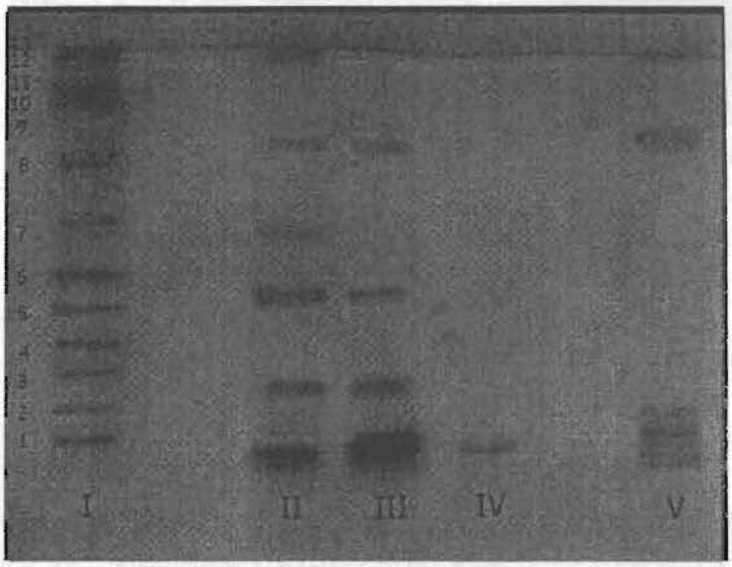

Figure 3. PAGE pattern of the crude venom of *M. gibbosus* and the fractions obtained from the HPLC purification of the venom by using C4 column first. Lane I corresponds the molecular weights of the known proteins whereas Lane II corresponds the crude venom; Lane III and Lane V, corresponds the G-50 fractions* and Lane IV, corresponds the active fraction from C4 column called M5. The relative mobility of the samples were then fitted to the curve to determine their molecular weights and it was determined that G-50 fraction consisted of at least 6 peptides with the molecular weights of 6300, 6400, 6600, 23000, 33000 and 64000 Da. In lane IV, M5 fraction consisted of one protein band with the molecular weights of 6500 Da.

(Lane I: 1.Aprotinin , 6500 Da; 2. α-Lactalbumin, 14200 Da; 3. Trypsin inhibitor, 20000 Da, 4. Trypsinogen, 24000 Da; 5. Carbonic anhydrase, 29000 Da; 6. Glyseraldehyde-3-phosphate dehydrogenase 36000 Da; 7. Ovalbumin, 45000 Da; 8.Glutamic dehydrogenase, 55000 Da; 9. Albumin, 66000 Da; 10.Fructose-6-phosphate kinase, 84000 Da; 11.Phosphorylase b, 97000 D; 12. β-galactosidase, 116000 Da; 13. Myosin, 205000 Da)

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* The crude venom of *M. gibbosus* was applied to gel filtration chromatography for another study (data not shown). Gel filtration chromatography was run using Sephadex G-50 fine column (1.6 x 100 cm) equilibrated with 20 mM ammonium acetate buffer, pH 4.7.

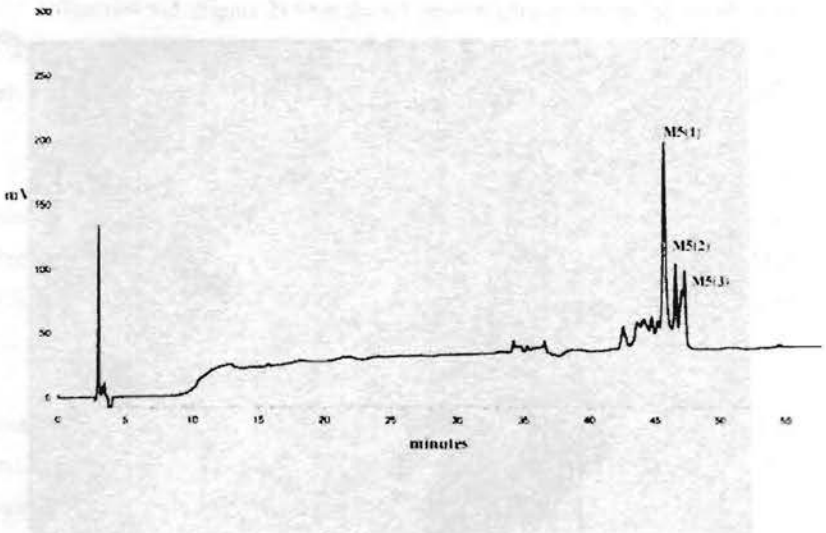

Figure 4. Separation of crude venom by RP-HPLC using a C18 column. The separation was established as explained in Materials and Methods section. Individual peaks, M5(1), M5(2) and M5(3) were collected, lyofilized and used for molecular weight analysis and bioassays.

Table 2. Toxicological profile of the C18 fractions of M5

[Toxic (T): Injected animals all showing signs of poisoning; Lethal (L): Injected animals dead; NT (Non-toxic)]

Fraction	Mice [a]	*M. domestica* larvae [b]
M5(1)	**L**	**T**
M5(2)	NT	NT
M5(3)	NT	NT

[a] i.c.v. injection ; 1.0 µL / mice

[b] postabdominal injection; 1.0 µL / 30 mg body weight

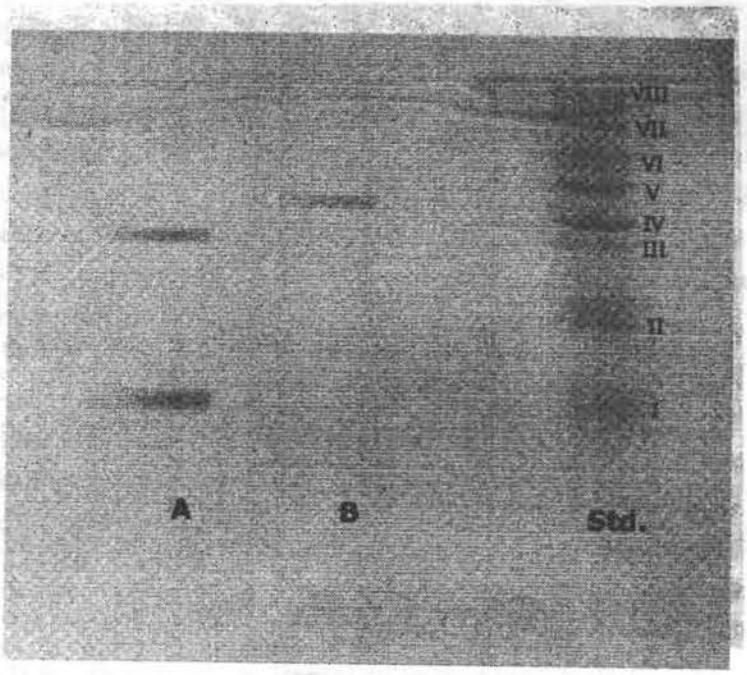

Figure 5. SDS-PAGE pattern of the toxic fraction obtained from HPLC seperation . Toxic fraction which was applied to the C-18 column was obtained as the toxic fraction M5(1) from the C4 column of M5 fraction. Lane Std., corresponds the molecular weights of the known proteins shown below; Lane A corresponds the peptides by the treatment of the sample with mercaptoethanol; Lane B corresponds the toxic peptide without treatment with mercaptoethanol.

(Std Lane: I. Aprotinin, 6500 Da; II. α-Lactalbumin, 14200 Da; III. Trypsin inhibitor soybean, 20000 Da; IV. Trypsinogen, bovine pancreas, 24000 Da; V. Carbonic anhydrase, bovine erythrocytes, 29000 Da; VI. Glyseraldehyde-3-phosphate dehydrogenase, rabbit muscle, 36000 Da ; VII. Ovalbumin, chicken egg, 45000 Da; VIII. Albumin, bovine serum, 66000 Da)

Discussion

During the last several years a large number of toxins have been isolated from various scorpion species [12,13,14] . The toxicity of scorpion venom is due to the neurotoxin(s) present in it. Scorpion venom is a rich source of polypeptide neurotoxins that effect ion channels specifically. These toxins are low molecular weight basic polypeptides consisting of single chains bound by disulphide bridges and each scorpion venom has neurotoxins.

Structural and pharmacological studies allowed to distinguish two main families of scorpion neurotoxins; the short-chain toxins (29 to 39 amino acid residues; three disulphide bridges) which are mainly acting on potassium channels[15], and the long-chain toxins (60 to 70 amino acid residues; four disulphide bridges) which act on voltage-gated sodium channels. In opposition to the interest of scientific research all over the world about the scorpions, in Turkey there are not enough studies such as purification and characterization of scorpion venoms. Scorpion species of Turkey and their distribution is tried to be determined. Although, scorpion fauna of Turkey has 13 species, this subject is studied only systematically not biochemically and/or physiologically.

In this study, the purpose was to obtain detailed information about biological characteristics of the scorpion Mesobuthus gibbosus, which has a wide range habitat in our country. Isolation of neurotoxic peptide from the scorpions, i.e. Mesobuthus gibbosus, has main applications. As summarized in the introduction part, identified peptides can be employed in the design and production of the anti-venom.

For the purification process, we prefer to use RP-HPLC, because this purification procedure, beginning with C4 column and then C18 column, should be considered as an appropriate tool for biochemical studies of scorpion venoms. This experimental approach could allow the isolation and characterization of neurotoxins available in less than 1 mg of venom [16]. According to the results, the molecular weights of the neurotoxic fragments belong to the *Mesobuthus gibbosus* venom was 6600 Da. The following step from this point of view will be the characterization of this component, such as amino acid sequence determination and mass spectrophotometric analysis and use these results as primary experimental design procedure for the other scorpion species in Turkey.

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NEW FLORISTIC RECORDS FOR A4, B1, B4, B5, B7 and C3 SQUARES IN FLORA OF TURKEY

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Abstract

In this paper 66 taxa are reported as new records from A4, B1, B4, B5, B7 and C3 squares in Turkish Flora.

Key words: New floristic records, Plant distribution, Flora, Turkey.

Introduction

Because of the great number of local floristic studies, scientific excursions and revisions have been performed on various part of Turkey, knowledge about Turkish plants distribution rapidly increases, so the number of new records is decreasing day by day. Since the distributions of the plant species have a dynamic face, with these activities mentioned above new distribution areas and as a result of this new square records come out. This paper is a contribution to this knowledge. *The Flora of Turkey and East Aegean Islands* (1, 2, 3) is not sufficient to check all the plant distribution records so chorology papers have to be checked Yıldırımlı' s archive and papers (4-17) helped us to find which species is new record and

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which is not. After this process 65 species and 1 subspecies are determined as new record for the squares A4, B1, B4, B5, B7 and C3.

Material and Methods

Plant materials were collected during the study on Flora of Kirmir Valley and other floristic excursions in Ankara, Burdur, Çanakkale, Kırşehir, Adıyaman, Nevşehir, Kayseri and Antalya province between 1975 and 2003. Specimens were identified according to *The Flora of Turkey and The East Aegean Islands* (1, 2, 3), and checked up carefully from the relevant papers about these squares (4-17). These species are given together in alphabetical order. Author names were written according to Brummitt and Powell (18). Phytogeographical regions and endemism of the taxa were evaluated according to *The Flora of Turkey and The East Aegean Islands* (1, 2, 3). Red Data Categories are given after the locality of species as threaten category (19, 20). Abbreviations used in the text are as follows: Ir.-Tur.: Irano-Turanian, Medit.: Mediterranean, E. Medit.: East Mediterranean, VU: Vulnerable; NT: Near Threatened, LC: Least Concern. Plant specimens are deposited at the Hacettepe University Department of Biology Herbarium in Ankara (HUB).

Results

Sixty-five species and 1 subspecies have been found as a new records for the A4, B1, B4, B5, B7 and C3 squares in Turkish Flora. The list of these species is given below:

ANACARDIACEAE

Pistacia vera L.

A4 ANKARA: Güdül, Çerçininkaya ridge, south-east side, Kirmir valley, agglomerate rocks, 730 m, 16/5/2001, **B. Tarıkahya** 1243. **A4** ANKARA: Güdül, around İn önü caves, Kirmir valley, agglomerate rocks, N 40° 13' 18.04"-E 32 °14.' 48.1", 680 m, 23/6/2002, **B. Tarıkahya** 1734. Ir-Tur element. (Culture).

This species was firstly collected from squares of "A" series. It's only known from the squares C 1, 4, 5, 6, 7, 8.

APIACEAE

Anthriscus caucalis M.Bieb.

A4 ANKARA: Güdül, near Bent, Kirmir valley, stream side, muddy, N 40° 12' 58"-E 33 °12' 44", 690 m, 30/6/2002, **B. Tarıkahya** 1772.

Torilis arvensis (Huds.) Link subsp. *elengata* (Hoffmanns. & Link) Cannon

C3 ANTALYA: İbradı, Altınbeşik Cave National Park, Behind of Aşağılar Fountain, Göğrecik Stream, 800 m, 7/6/2003, **B. Mutlu** 8503.

ASTERACEAE

Echinops pungens Trautv. var. *pungens*

A4 ANKARA: Güdül, Balıklar, below Alagöz hill, Kirmir valley, agglomerate rocks, N 40° 13' 10.5"-E 32 ° 13' 37.5", 720 m, 23/6/2001, **B. Tarıkahya** 1301. **A4** ANKARA: Güdül, around İn önü caves, Kirmir valley, agglomerate rocks, N 40° 13' 18.04"-E 32 °14.' 48.1", 680 m, 11/8/2001, **B. Tarıkahya** 1323. Ir-Tur element.

Pulicaria odora (L.) Rchb.

A4 ANKARA: Güdül, between Güdül-Yeşilöz, around useless bridge, Kirmir valley, agglomerate rocks, N 40° 14' 39.2"-E 32 °15' 25.2", 750 m, 23/6/2002, **B. Tarıkahya** 1699. Medit. element.

Tragopogon latifolius Boiss. var. *angustifolius* Boiss.

B1 ÇANAKKALE: Truva National Park, Papaz beach, 10 m, 27/4/2001, **B. Mutlu** 6423. Ir.-Tur. element.

BORAGINACEAE

Heliotropium hirsutissimum Grauer

A4 ANKARA: Güdül, around İn önü caves, Kirmir valley, agglomerate rocks, N 40° 13' 18.04"-E 32 °14.' 48.1", 680 m, 11/8/2001, **B. Tarıkahya** 1324. E.Medit. element.

BRASSICACEAE

Brassica tournefortii Gouan

B5 KIRŞEHİR: Seyfe Lake, lake side, 970 m, 4/7/1998. **B. Mutlu** 3831.

Alyssum strictum Willd.

A4 ANKARA: Güdül, Karamanağacı, Kirmir valley, steppe, near agriculture fields, N 40° 13.01' 1"-E 32 °14.33' 0", 754 m, 10/5/2001, **B. Tarıkahya** 1142. Ir-Tur element.

Erophila verna (L.) Chevall. subsp. *praecox* (Stev.) Walters

A4 ANKARA: Güdül, around İn önü caves, Kirmir valley, agglomerate rocks, N 40° 13' 18.04"-E 32 °14.' 48.1", 680 m, 10/4/2001, **B. Tarıkahya** 1065, **S. Erik, B. Mutlu**.

E. verna (L.) Chevall. subsp. *spathulata* (Lang) Walters

A4 ANKARA: Güdül, around İn önü caves, Kirmir valley, agglomerate rocks, N 40° 13' 18.04"-E 32 °14.' 48.1", 680 m, 27/4/2002, **B. Tarıkahya** 1466.

CARYOPHYLLACEAE

Dianthus capitatus Balb. ex DC.

A4 ANKARA: Güdül, Yeşilöz town, İnbaşı ridge, Kirmir valley, agglomerate rocks, N 40° 15' 17.0"-E 32 °15' 56.2", 760 m, 23/6/2002, **B. Tarıkahya** 1326. Euro-Sib. element.

D. cinnamomeus Sibth.&Sm.

B4 ANKARA: Gölbaşı, near of Ahiboz Village, road side, 970 m, 16/8/2001, **B. Mutlu** 7834. Medit. element.

Velezia pseudorigida Hub.-Mor.

A4 ANKARA: Güdül, near Bent, Kirmir valley, agglomerate rocks, N 40° 12' 58"-E 33 °12' 44", 690 m, 30/6/2002, **B. Tarıkahya** 1786. **A4** ANKARA: Güdül, Çerçininkaya ridge, south-east side, Kirmir valley, agglomerate rocks, 730 m, 16/5/2001, **B. Tarıkahya** 1272. Endemic. Threaten category: VU. E. Medit element.

CHENOPODIACEAE

Chenopodium murale L.

A4 ANKARA: Güdül, Yeşilöz town, İnbaşı ridge, Kirmir valley, agglomerate rocks, N 40° 15' 17.0"-E 32 °15' 56.2", 760 m, 7/9/2002, **B. Tarıkahya** 1914.

C. sosnowskyi Kapeller

A4 ANKARA: Güdül, towards Köprübaşı ridge, Kirmir valley, meadow, 700 m, 7/9/2002, **B. Tarıkahya** 1931. Ir-Tur element.

Petrosimonia nigdeensis Aellen

C3 BURDUR: Burdur lake side, 15/9/1994, **İ. Kiziroğlu**. Endemic. Threaten category: NT.

CUSCUTACEAE

Cuscuta campestris Yunck.

A4 ANKARA: Güdül, around useless bridge between Güdül-Yeşilöz, Kirmir valley, on Fabaceae, N 40° 14' 39.2"-E 32 °15' 25.2", 750 m, 28/7/2002, **B. Tarıkahya** 1873. **A4**

ANKARA: Güdül, near Bent, Kirmir valley, N 40° 12' 58"-E 33 °12' 44", 690 m, 30/6/2002, **B. Tarıkahya** 1775.

C. monogyna Vahl. subsp. *esquamata* (Engelm.) Plitmann

A4 ANKARA: Güdül, between Güdül-Yeşilöz, around useless bridge, Kirmir valley, agglomerate rocks, N 40° 14' 39.2"-E 32 °15' 25.2", 750 m, 7/9/2002, **B. Tarıkahya** 1928.

CYPERACEAE

Carex acuta L.

A4 ANKARA: Güdül, between Güdül-Yeşilöz, around bridge, Kirmir valley, stream side, N 40° 14' 11.09"-E 32 °15' 44.4", 750 m, 23/6/2002, **B. Tarıkahya** 1723. **A4** ANKARA: Güdül, around İn önü caves, Kirmir valley, stream side, N 40° 13' 18.04"-E 32 °14.' 48.1" 680 m, 10/4/2001, **B. Tarıkahya** 1076, **S. Erik, B. Mutlu**. Euro-Sib. element.

C. nigra (L.) Reichard subsp. *dacica* (Heuff.) Soó

A4 ANKARA: Güdül, between Güdül-Yeşilöz, around useless bridge, Kirmir valley, stream side, N 40° 14' 39.2"-E 32 °15' 25.2" 750 m, 2/6/2002, **B. Tarıkahya** 1555. Euxine element.

Cyperus serotinus Rottb.

A4 ANKARA: Güdül, towards Köprübaşı ridge, Honda gardens, Kirmir valley, stream side, 700 m, 7/9/2002, **B. Tarıkahya** 1930.

FABACEAE

Medicago orbicularis (L.) Bartal.

A4 ANKARA: Güdül, Alagöz hill, steppe, N 40° 12' 37.0"-E 32 ° 13' 41.3", 770 m, 11/5/2002, **B. Tarıkahya** 1529.

Trifolium fragiferum L. var. *fragiferum*

A4 ANKARA: Güdül, Çerçininkaya ridge, south-east side, Kirmir valley, agglomerate rocks, 730 m, 16/5/2001, **B. Tarıkahya** 1240.

T. sylvaticum Gérard ex Loisel

A4 ANKARA: Güdül, Yeşilöz town, İnbaşı ridge, Kirmir valley, agglomerate rocks, N 40° 15' 17.0"-E 32 °15' 56.2", 760 m, 23/6/2002, **B. Tarıkahya** 1604.

Trigonella balansae Boiss.&Reut.

A4 ANKARA: Güdül, Karamanağacı, Kirmir valley, steppe, near agriculture fields, N 40° 13.01' 1"-E 32 °14.33' 0", 754 m, 10/5/2001, **B. Tarıkahya** 1207. Medit. element.

Vicia articulata Hornem.

A4 ANKARA: Güdül, between Güdül-Yeşilöz, around useless bridge, Kirmir valley, agglomerate rocks, N 40° 14' 39.2"-E 32 °15' 25.2", 750 m, 11/5/2002, **B. Tarıkahya** 1492. This species was firstly collected from squares of "A" series. It's only known from the squares B 1, 2, 4, C 1, 2.

V. villosa Roth. subsp. *dasycarpa* (Ten.) Cav.

A4 ANKARA: Güdül, Çerçininkaya ridge, south-east side, Kirmir valley, agglomerate rocks, 730 m, 16/5/2001, **B. Tarıkahya** 1225.

GERANIACEAE

Geranium divaricatum Ehrh.

A4 ANKARA: Güdül, Çerçininkaya ridge, south-east side, Kirmir valley, agglomerate rocks, 730 m, 16/5/2001, **B. Tarıkahya** 1219.

JUNCACEAE

Juncus sparganiifolius Boiss. & Kotschy ex Buchenau

B1 ÇANAKKALE : Truva National Park, between Yenikumkale-Orhaniye village, 100 m, 24/5/2001, **B. Mutlu** 6706. Endemic. Threaten category: LC. E. Medit. element.

LAMIACEAE

Ajuga chamaepitys (L.) Schreb. subsp. *chia* (Schreb.) Arcang. var. *ciliata* Briq.

C3 ANTALYA: İbradı, Altınbeşik Cave National Park, around of İnönü Hill, 850 m, 7/6/2003, **B. Mutlu** 8334.

Lamium pisidicum R.R.Mill. .

A4 ANKARA: Güdül, around İn önü caves, Kirmir valley, agglomerate rocks, N 40° 13' 18.04"-E 32 °14.' 48.1", 680 m, 21/4/2002, **B. Tarıkahya** 1430. Endemic. Threaten category: NT. Medit. element.

This species was firstly collected from squares of "A" series. It's only known from the squares B 1, 2, 3, C 3, 4.

Marrubium globosum subsp. *globosum* Montbret & Aucher ex Benth.

A4 ANKARA: Güdül, Çerçininkaya ridge, south-east side, Kirmir valley, agglomerate rocks, 730 m, 16/5/2001, **B. Tarıkahya** 1252c. Endemic. Threaten category: LR. Ir-Tur element.

Scutellaria rubicunda Hornem. subsp. *brevibracteata* (Stapf) J.R.Edm.

A4 ANKARA: Güdül, Yeşilöz town, İnbaşı ridge, Kirmir valley, agglomerate rocks, N 40° 15' 17.0"-E 32 °15' 56.2", 760 m, 23/6/2002, **B. Tarıkahya** 1639. Endemic. Threaten category: LR. E.Medit. element.

This species was firstly collected from squares of "A" series. It's only known from the squares C 2, 4, 6.

Sideritis leptoclada O.Schwarz & P.H.Davis

C3 Olimpos Seashore National Park, around of Naturland Hotel, road side, 50 m, 12/6/2000. **B. Mutlu** 5977. Endemic. Threaten category: NT. E. Medit. element.

Stachys cretica L. subsp. *bulgarica* Rech.fil.

B1 ÇANAKKALE: Truva National Park, Papazdamı Hill, 100 m, 28/6/2001. **B. Mutlu** 7507.

S. woronowii (Schisch. ex Grossh.) R.Mill

A4 ANKARA: Güdül, Karamanağacı, Kirmir valley, steppe, near agriculture fields, N 40° 13.01' 1"-E 32 °14.33' 0", 754 m, 10/5/2001, **B. Tarıkahya** 1157. Ir-Tur element.

LILIACEAE

Ornithogalum comosum L.

B1 ÇANAKKALE: Truva National Park, around of Sivritepe, 50 m, 27/4/2001, **B. Mutlu** 6443.

Tulipa humilis Herb.

B7 ADIYAMAN: Yazıbaşı, Kötigeri, 1200-1500 m, 27/5/1981.

MALVACEAE

Abutilon theophrastii Medik.

A4 ANKARA: Güdül, near Bent, Kirmir valley, dry brook, N 40° 12' 58"-E 33 °12' 44", 690 m, 29/672003, **B. Tarıkahya** 2054.

PAPAVERACEAE

Glaucium acutidentatum Hausknecht & Bornm.

A4 ANKARA: Güdül, Yeşilöz town, İnbaşı ridge, Kirmir valley, agglomerate rocks, N 40° 15' 17.0"-E 32 °15' 56.2", 760 m, 28/7/2002, **B. Tarıkahya** 1866. Endemic. Threaten category: LR. Ir-Tur element.

POACEAE

Elymus hispidus (Opiz) Melderis subsp. *hispidus*

B1 ÇANAKKALE : Truva National Park, Papazdamı Hill, 50 m, 28/6/2001, **B. Mutlu** 7496.

Eragrostis minor Host.

B1 ÇANAKKALE : Truva National Park, between Truva ruins and park entrance, 50 m, 28/6/2001, **B. Mutlu** 7478.

Hordeum distichon L.

B4 ANKARA: Çankaya, roadsides between Beytepe-Bilkent, 15/6/1975, **S. Erik** 1900. (Culture)

Phleum bertolonii DC.

C3 ANTALYA: İbradı, Altınbeşik Cave National Park, around of İnönü Hill, 850 m, 7/6/2003, **B. Mutlu** 8299.

Poa compressa L.

C3 ANTALYA: İbradı, Altınbeşik Cave National Park, around of İnönü Hill, 850 m, 7/6/2003, **B. Mutlu** 8368.

Secale montanum Guss

B1 ÇANAKKALE : Truva National Park, around of Kalafatlı Village, 50 m, 24/6/2001, **B. Mutlu** 6773

Trisetaria loeflingiana (L.) Paunero

A4 ANKARA: Güdül, Çerçiñinkaya ridge, south-east side, Kirmir valley, agglomerate rocks, 730 m, 16/5/2001, **B. Tarıkahya** 1221. Euro-Sib. element.

Vulpia ciliata Dumort. subsp. *ciliata*

A4 ANKARA: Güdül, between Güdül-Yeşilöz, around useless bridge, Kirmir valley, agglomerate rocks, N 40° 14' 39.2"-E 32 °15' 25.2", 750 m, 2/6/2002, **B. Tarıkahya** 1554.

POLYGONACEAE

Polygonum setosum Jacq.

A4 ANKARA: Güdül, between Güdül-Yeşilöz, around useless bridge, Kirmir valley, agglomerate rocks, N 40° 14' 39.2"-E 32 °15' 25.2", 750 m, 23/6/2002, **B. Tarıkahya** 1687. Ir-Tur element.

PORTULACACEAE

Portulaca oleracea L.

A4 ANKARA: Güdül, near Bent, Kirmir valley, agglomerate rocks, N 40° 12' 58"-E 33 °12' 44", 690 m, 30/6/2002, **B. Tarıkahya** 1769.

POTAMOGETONACEAE

Potamogeton nodosus Poir.

A4 ANKARA: Güdül, near Bent, Kirmir valley, in stream, N 40° 12' 58"-E 33 °12' 44", 690 m, 28/7/2002, **B. Tarıkahya** 1905.

RANUNCULACEAE

Ranunculus cornutus DC.

A4 ANKARA: Güdül, Karamanağacı, Kirmir valley, steppe, near agriculture fields, N 40° 13.01' 1"-E 32 °14.33' 0", 754 m, 10/4/2001, **B. Tarıkahya** 1002, **S. Erik, B. Mutlu**. **A4** ANKARA: Güdül, Çerçininkaya ridge, south-east side, Kirmir valley, agglomerate rocks, 730 m, 16/5/2001, **B. Tarıkahya** 1248.

R. paludosus Poir.

A4 ANKARA: Güdül, Karamanağacı, Kirmir valley, steppe, near agriculture fields, N 40° 13.01' 1"-E 32 °14.33' 0", 754 m, 27/4/2002, **B. Tarıkahya** 1432.

RHAMNACEAE

Zizyphus jujuba Mill.

A4 ANKARA: Güdül, Yeşilöz entrance from Çeltikçi road, Kirmir valley, agglomerate rocks, N 40° 15' 22.9"-E 32 °16' 2.7", 760 m, 17/6/2003, **B. Tarıkahya** 2012.

ROSACEAE

Amygdalus webbii Spach.

A4 ANKARA: Güdül, Karamanağacı, Kirmir valley, steppe, near agriculture fields, N 40° 13.01' 1"-E 32 °14.33' 0", 754 m, 10/4/2001, **B. Tarıkahya** 1007, **S. Erik, B. Mutlu**. E.Medit. element.

Cotoneaster morulus Pojark.

A4 ANKARA: Güdül, Karamanağacı, Kirmir valley, steppe, near agriculture fields, N 40° 13.01' 1"-E 32 °14.33' 0", 754 m, 10/5/2001, **B. Tarıkahya** 1193.

Pyrus elaeagnifolia Pall. subsp. *kotschyana* (Boiss.) Browicz

A4 ANKARA: Güdül, Balıklar, below Alagöz hill, Kirmir valley, agglomerate rocks, N 40° 13' 10.5"-E 32 ° 13' 37.5", 720 m, 30/6/2002, **B. Tarıkahya** 1835.

RUBIACEAE

Asperula setosa Jaub. & Spach

A4 ANKARA: Güdül, near Bent, Kirmir valley, agglomerate rocks N 40° 12' 58"-E 33 °12' 44", 690 m, 11/5/2002, **B. Tarıkahya** 1510. Ir-Tur element.

SCROPHULARIACEAE

Digitalis cariensis Boiss. ex Jaub.& Spach.

A4 ANKARA: Güdül, Yeşilöz town, İnbaşı ridge, Kirmir valley, agglomerate rocks, N 40° 15' 17.0"-E 32 °15' 56.2", 760 m, 2/6/2002, **B. Tarıkahya** 1579. **A4** ANKARA: Güdül, around İn önü caves, Kirmir valley, agglomerate rocks, N 40° 13' 18.04"-E 32 °14.' 48.1", 680 m, 23/6/2002, **B. Tarıkahya** 1760. Endemic. Threaten category: LR. E.Medit. element.

Scrophularia catariifolia Boiss.&Heldr.

B5 NEVŞEHİR: Göreme, volcanic tuff, 28/6/1980, **S. Erik** 3852. Ir-Tur element.

Verbascum flavidum (Boiss.) Freyn&Bornm.

B5 KAYSERİ: Develi, Saraycık village, around mine, 1700 m, *P. nigra* forest, 3/6/1981, **S. Erik** 3178. Euro-Sib. element.

V. syriacum F.C.Schrad.

B1 ÇANAKKALE : Truva National Park, around of Kalafatlı Village, 50 m, 24/5/2001, **B. Mutlu** 6770.

This species was firstly collected from squares of "B" series. It's only known from the squares C 2.

V. sinuatum L. var. *adenosepalum* Murb.

B1 ÇANAKKALE : Truva National Park, Papazdamı Hill, 50 m, 28/6/2001, **B. Mutlu** 7503.

THYMELACEAE

Thymelaea passeriana (L.) Coss.&Germ

B1 ÇANAKKALE : Truva National Park, around of Orhaniye gunner battalion, 10 m, 28/6/2001, **B. Mutlu** 7529.

VALERIANACEAE

Valerianella locusta (L.) Laterr.

A4 ANKARA: Güdül, Karamanağacı, Kirmir valley, steppe, near agriculture fields, N 40° 13.01' 1"-E 32 °14.33' 0", 754 m, 10/4/2001, **B. Tarıkahya** 1010, **S. Erik, B. Mutlu**.

Discussion

43 species and 1 subspecies for A4, 10 species for B1, 2 species for B4, 3 species for B5, 1 species for B7 and 6 for C3 squares were recorded for the first time. *Pistacia vera, Vicia articulata, Lamium pisidicum* and *Scutellaria rubicunda* were firstly collected in A series and *Verbascum syriacum* was firstly collected in B series of Turkish square system (1).

The floristic summary is given in table 1. Eight endemic species, 10 Ir.-Tur. elements, 11 Medit. elements and 4 Euro-Sib. elements were determined in these studies. Endemic species were evaluated according to IUCN risk categories. One species is placed in the VU, while 3 species in the NT category and 5 species are put in the LC.

Table 1. Floristic summary about this study.

Species	65
Subspecies	1
Endemic species	9
Ir.-Tur. elements	10
Medit elements	4
E. Medit elements	7
Euro.-Sib. elements	3
Euxin	1
VU	1
NT	3
LC	5

Phytogeographic regions are suggested for 2 taxa in Table 2. Phytogeographic position of *Dianthus cinnamomeus* Sibth. & Sm. and *Lamium pisidicum* R.R.Mill have not been indicated in Flora of Turkey and The East Aegean Islands (1, 2, 3) and other relevant papers. These species are very common in the Mediterranean regions but also found in the Gölbaşı and Kirmir Valley. Although the Gölbaşı and Kirmir Valley are in the central Anatolia, the Mediterranean effect is seen in both areas. Therefore, we suggest that *Dianthus cinnamomeus* and *Lamium pisidicum* are Medit. elements.

Table 2. Phytogeograpic regions recommended for the taxa

Species	Distribution area		Endemism State	Suggested phytogeographic regions for species
	in Turkey	outside Turkey		
Dianthus cinnamomeus	A4, B4, C3, C4, C5	Greece, Cyprus	-	Medit.
Lamium pisidicum	A4, B1, B2, B3, C3, C4		Endemic	Medit.

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APPLICATION OF THE *Mat*K GENE SEQUENCES TO SOME WILD WHEAT SPECIES FROM TURKEY[1]

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Abstract

The 3' region of the *mat*K gene from 16 individuals belonging to four species, *Triticum monococcum* var. *boeoticum, Triticum urartu, Triticum aestivum* and *Triticum dicoccoides*, is used to investigate their evolutional relationships and the effects of ecological and climatical factors on the samples collected from different localities of Central and South-East regions of Anatolia. When sequenced regions were compared with *mat*K region of *Triticum aestivum* taken from the GeneBank, species can be divided into two groups. There are base differences at only two positions between these two groups.

Keywords: *Mat*K gene, sequence analysis, phylogenie, wheat.

Introduction

Wheat is the earliest domesticated and the most widely cultivated food crop in all of the cereals. It will continue its importance of being the main food source of human beings in the future as if in today and in the past (1). Wheat turned the investigators towards new researches for increasing the yield and the quality of products, because of its valuable

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properties. Plant breeding studies in which the molecular techniques were used have accelerated during the last fifty years in worldwide. The molecular studies about the improvement of wheat brought out an important problem, a drastic erosion of genetic resource of wheat. The selection procedures for improvement of wheat have seriously narrowed the genetic pool. Therefore genetic homogenity has also increased due to modern pure-breeding practice. The main aim of plant breeding studies is to increase the yield and quality and also to develope more resistant plants.

Recently, several molecular approaches have been used for analyzing both genetic diversity and phylogenetic relationship of plants. Among these approaches, comparative DNA sequencing has become a widespread tool for investigating phylogenetic relationships, because DNA sequencing is relatively fast, convenient, and offers a large data set of discrete characters. Comparative studies of the nucleotide sequences provide a means for investigating phylogenetic relationships over a wide range of taxonomic levels. Three types of molecular characters are used in phylogenetic studies: nucleotide substitutions, rearrangements and insersion/deletions (indels) (2). Coding and spacer regions of a number of chloroplast, mitochondrial and nuclear genes are used for phylogenetic purposes, especially, nuclear ribosomal DNA subunits (18S, 5.8S, 26S, ITS1 and ITS2) (3,4,5), *rbc*L, *mat*K and *psb*A. In a study of barley and rice, comparing the sequences of *rbc*L, *mat*K, *psb*A and *rps*4, the highest degree of nucleotide substitution was detected for *mat*K (Table 1) (6). It has also been reported that *mat*K gene has the highest overall nucleotide substitution rate among the 20 genes used in molecular systematics (7). Therefore *mat*K gene has proven to be a useful source of molecular characters for phylogenetic studies. The gene (previously designed ORFK or ORFK509) is approximately 1500 base pairs in length (Figure 1). It is a maturase coding gene embedded within a 2600 base pairs intron that interrupts the two *trn*K exons, on the large single-copy section adjecent to the inverted repeat (8) (Figure 2). A homology search about *mat*K region has shown that the positions of 102 amino acids at the carboxyl terminus (at amino acid positions 369-471) are structurally related to portions of maturase-like polypeptide and might involve in splicing group II intron (9). The presence of the gene in the parasitic *Epifagus* that lost approximately 65% of its chloroplast genes, indicates the functional significance of the *matK* gene in plants (10).

Table 1. Comparison of four chloroplast genes from rice and barley for nucleotide variation, G+C content, and transition-transversion ratios (ns:nv).

Gene	Similarity	Length	G+C (%)	Variable site	%	ns:nv
rbcL	94.28	1725	40.8	95	5.72	2.276
psbA	97.27	1062	42.0	29	2.73	3.833
rps4	96.26	588	36.9	28	3.74	1.200
matK	91.06	1560	33.9	135	8.94	1.250

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1    ATGGAAAAAT TCGAAGGGTA TTCAGAAAAA CATAAATCTC GTCAACAATA CTTCGTCTAC
61    CCACTTCTCT TTCAGGAATA TATTTATGCA TTTGCCCACG ATTATGGATT AAATGGTTCT
121   GAACCTGTGG AAATAGTTGT TAGTTGTAAT AACAAGAAAT TTAGTTCACT ACTTGTGAAA
181   CGTTTAATTA TTCGAATGTA TCAGCAGAAT TTTTTGGATA ACTCGGTTAA TCATCCTAAC
241   CAAGATCGAT TATTGGATTA CAAAAATTAT TTTTATTCTG AGTTTTATTC TCAGATTCTA
301   TCTGAAGGGT TTGCGATCGT TGTGGAAATC CCATTCTCGC TACGGGAATT ATTTTGTCCG
361   AAAGAAAAAG AAATACCAAA GTTTCAGAAT TTACGCTCTA TTCATTCAAT ATTTCCTTTT
421   TTGAAAGACA AATTTTTGCA TTTGGATTAT CTATCACATA TAGAAATACC CTATCCTATC
481   CATTTGGAAA TCCTGGTTCA ACTCCTTCAA TACCGTATCC AAGATGTTCC ATCTTTGCAT
541   TTATTGCGAT TCTTTCTCAA CTACTATTCG AATTGGAATA GTTTTATTAC TTCAATGAAA
601   TCCATTTTTT TTTTTCAAAA AGAAAATAAA AGACTATTTC GATTCCTATA TAACTCTTAT
661   GTATCAGAAT ATGAATTTTT TTTGTTGTTT CTTCGTAAAC AATCTTCTTG CTTACCATTA
721   GCATCTTCTG GAACTTTTCT GGAACGAATC CACTTTTCTA GGAAGATGGA ACATTTTGGG
781   ATAATGTACC CTGGTTTTTC TCGGAAAACC CTATGGTTCT TTATGGATCC TCTTATGCAT
841   TATGTTCGAT ATCAAGGAAA GGCAATTCTT GCATCAAAAG GCACTTTTTT TTTGAAGAAG
901   AAATGGAAAT GCTACCTTAT CAATTTATGG CAATATTATT TCTGTTTTTG GACTCAGCCG
961   CGAAGAATCC ATATAAACCA ATTAGCAAAC TCTTGCTTCG ATTTTATGGG GTACCTTTCA
1021  AGTGTACCAA AAAGTTCTTT GTTAGTAAGG AATCAAATGC TGGAGAATTC ATTTCTAATA
1081  GATACTCGAA TGAAAAAATT CGATACCATA GTCCCCGCTA CTCTCCTCAT AGGATACTTA
1141  TCAAAAGCTC AATTTTGTAC TGGATCGGGG CATCCTATTA GTAAACCCAT TTGGACAGAT
1201  TTATCAGATT GGGATATTCT TGATCGATTT GGTCGGATAT GTAGAAATCT TTTTCATTAT
1261  CATAGTGGAT CTTCGAAAAA ACGGACTTTG TATCGACTAA AGTATATACT TCGACTTTCA
1321  TGCGCTAGAA CTTTAGCTCG TAAACATAAA AGCACGGTAC GAACTTTTAT GCAACGGTTG
1381  GGTTCGGCAT TTTTAGAAGA ATTTTTTATG GAAGAAGAGC AAGTTTTTTC TTTGATGTTC
1441  ACCAAAACAA CTCTTTTTTC TTTCTGTGGA TCACACACTG AGCGTATTTG GTATTTGGAT
1501  ATTATACGTA TCAATGACCT GGTCAACCCT CTTAATTAA
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Figure 1. *Mat*K sequence of *T. aestivum* from GeneBank (Accession number: AF164405)

In the present study, our goals are to resolve evolutionary relationship between *T. aestivum, T. dicoccoides, T. monococcum* var. *boeoticum* and *T. urartu* and to investigate the effects of ecological and climatical factors on the samples collected from different localities by comparing their *mat*K sequences.

Materials and Methods

a. Plant material

16 individuals belonging to four species of *Triticum* representing 3 genomes were studied. The names and genomic constitutions are listed in Table 2. They were collected from different ecological and climatical locations of Turkey (Table 3). The seed samples were germinated and grown in plant growth chamber.

Table 2. Wheat species used in this study

Name	Abbreviation	Genome Symbole	2n
Triticum monococcum var. *boeoticum*	TRMB	AA	14
Triticum urartu	TRUR	AA	14
Triticum dicoccoides	TRDI	AABB	28
Triticum aestivum	TRAE	AABBDD	42

Table 3. Ecological and climatical data of the samples used in the this study. The samples belonging to these species were collected from Central and South-East regions of Anatolia.

Species	Location	Latitute/ Longitute	Altitude (m)	Mean temperature(°C) Annual	August	January	Mean annual rainfall (mm)	Mean humidity at 14.00 (%)	*Soil type
TRAE-1	Balışeyh-Kırıkkale	39°.59'N 31°51'E	1300	10.3	21.8	-1.1	397.3	42	B
TRMB-2	Beytepe-Ankara	39°.52'N 32°44'E	900	11.2	22.3	-0.6	402.2	48.2	C
TRMB-3	Hilvan-Ş.Urfa (2,5 km)	37°.33'N 38°55'E	590	15.9	29.7	3.7	469.7	37	RB
TRMB-4	Viranşehir-Botaş(14 km)	37°.20'N 39°46'E	630	17.3	29.7	4.7	372	40	B
TRMB-5	Haymana-Ankara	39°37'N 32°40'E	1100	9.3	18.4	-2	413.6	59	RB
TRMB-6	Hilvan-15 km NW (Ş.Urfa)	37°.45'N 38°.46'E	575	15.9	29.7	3.7	469.7	37	RB
TRUR-7	Oğuzeli-Ekizkoyun (G.antep)	36°.52'N 37°.30'E	650	15.3	27.3	3.5	423.8	38	RB
TRUR-8	Ş.Urfa-Suruç (30 km)	37°.04'N 38°.31'E	670	18.1	31.1	5.4	305	38.3	RB
TRUR-9	Hilvan-Diyarbakır (14 km)	37°.36'N 39°.07'E	600	15.9	29.7	3.7	469.7	37	RB
TRUR-10	Karamuz Village (Diyarbakır)	37°.46'N 40°.14'E	720	15.7	30.2	1.7	261.8	41.9	B
TRUR-11	Ağızhan Village (Hilvan-Ş.Urfa)	37°.26'N 38°55'E	600	15.9	29.7	3.7	469.7	37	RB
TRDI-12	Siverek-Diyarbakır (2 km)	37°.45'N 39°.25'E	910	17.2	29.8	3.3	349.2	42	B
TRDI-13	Aşağıkarabahçe Village (Diyarbakır)	37°.48'N 39°.44'E	1100	15.7	30.2	1.7	261.8	41.9	B
TRDI-14	Karamuz Village-Ovabağ(8 km)	37°.46'N 40°.08'E	810	15.7	30.2	1.7	261.8	41.9	B
TRDI-15	Viranşehir-Botaş (10 km)	37°.20'N 39°.46'E	630	17.3	29.7	4.7	372	40	B
TRDI-16	Siverek-Diyarbakır (40 km)	37°.54'N 39°.52'E	900	17.2	29.8	3.3	349.2	42	B

*B: Basaltic; C: Calcereous; Rb: Red-brown; RM: Red Mediterranean

b. DNA extraction and amplification of *mat*K region

Extraction of DNA from fresh leaf tissues followed the procedure of Ma and Sorrels (11). Leaf tissues were ground in liquid N, and DNA extracted in extraction buffer (0.1 M Tris-HCl pH 8.0, 50 mM EDTA pH 8, 0.5 M NaCl, 12.5 gL^{-1} SDS). Extracted DNA was precipitated with 950 gL^{-1} alcohol and washed with 700 gL^{-1} alcohol three times. After drying, DNA was dissolved in Tris-EDTA pH 8.0 (10 mM Tris-HCl, 1 mM EDTA). Since the *mat*K gene is nested between the two exons of the *trn*K gene that contain conservative site for amplification, we selected primers for amplifying the whole *trn*K gene. We used two primers, MG1 (Forward) (5'- CTA CTG CAG AAC TAG TCG GAT GGA GTA GAT -3') and MG15 (Reverse) (5'- ATC TGG GTT GCT AAC TCA ATG-3'), located in the *trn*K 5' and 3' exons,

respectively, for PCR amplification (Figure 3). The amplified products were used as templates in sequencing reactions. For the PCR amplification, each reaction mixture (100 µL) contains 10 µL of 10XPCR reaction buffer (Promega), 1.5 mM magnesium chloride, 250 mM dNTPs, 40 µM of each of the two primers, 0.5 µL (2.5 units) of Taq DNA polymerase (Promega), 5 µL of genomic DNA templatc (~ 50 ng/µL). Amplification was carried out in a Biometra T-Personal thermocycler. The firts cycle was 96 °C for 1 min 30 s, 64 °C for 1 min, and 72 °C for 3 min, followed by 34 cycles of 95 °C for 30 s, 64 °C for 1 min, 72 °C for 5 min (6, 12). PCR products were electrophoresed in 1% agarose gels and visualized on U.V. lamb.

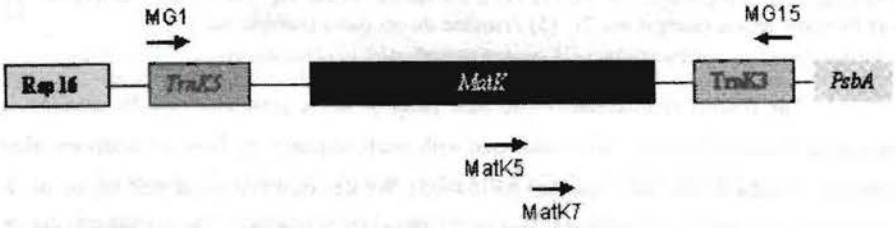

Figure 2. Localization of PCR and sequencing primers.

c. DNA sequencing

Double stranded PCR products were sequenced directly using the dideoxy chain-termination method (13). Sequenase Version 2.0 PCR Product Sequencing Kit (USB Corp.) was used to do sequencing reactions according to the instruction provided by the manufactures. MatK5 (5'- GGA TCC TTT CAT GCA TT-3') and MatK7 (5'- GTA TTA GGG CAT CCC ATT-3') were used as internal sequencing primers. Primers were obtained from Steele and Vilgalys (14). Localizations of the primers on genome were shown in Figure 2. The sequencing reaction products labeled radioactively were run on 6% polyacrylamide sequencing gel in 1XTBE buffer at 70 W (constant power) and exposed to Kodak Biomax films for 1-3 days.

Results and Discussion

The region which is approximately 2538 bp length in chloroplast genome of wheat was amplified by using polymerase chain reaction. This region is located among two introns of *trn*K gene. The PCR results are given in Figure 3.

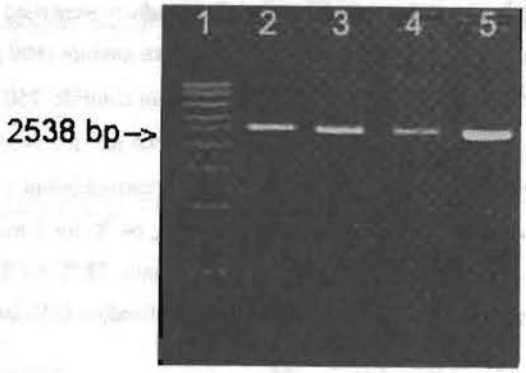

Figure 3. The PCR results of four species on agarose gel. (1) 1 kb DNA Ladder (Sigma), (2) *Triticum aestivum* (sample no. 1), (3) *Triticum monococcum* var. *boeoticum* (sample no. 2), (4) *Triticum urartu* (sample no. 7), (5) *Triticum dicoccoides* (sample no. 12).

The portion approximately 600 base pairs of *mat*K gene was read by sequencing reactions. Sequenced regions were compared with *mat*K sequence of *Triticum aestivum* taken from the Genebank (accession number AF164405). We detected that sequenced region of *T. aestivum* was entirely identical with that of *T. dicoccoides* (group1). On the other hand, *T. monococcum var. boeoticum* and *T. urartu* were entirely identical with each other in the respect of this region (group2). This is the result that we expect, because *T. monococcum var. boeoticum* and *T. urartu* have A genome and 14 chromosomes. We found base differences at only two positions between the two groups. This ratio of polymorphism which we found between the four wheat species are very low as unexpected. Cytosine is changed to adenine at position 1028 for *T. monococcum var. boeoticum* and *T. urartu* (Figure 4). This difference creates an amino asid change between the two groups, so at the position 343 of maturase gene, glutamine takes place instead of proline in group1 (Table 4). Cytosine is change to thymine at position 1323 for *T. monococcum var. boeoticum* and *T. urartu*. This difference causes a change in codon TG**T** into codon TG**C** but it does not create any amino acid change at the position 441 of maturase gene, because both of them code the same amino acid, cysteine (Table 4). The effect of these base changes on the function of this protein are not known yet.

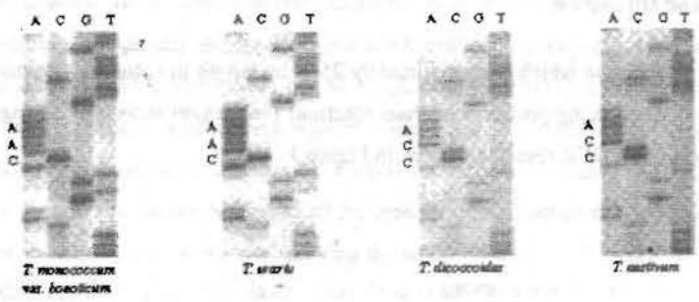

Figure 4. Base differencies at position 1028.

Table 4. Base changes in sequenced region of *mat*K gene. Abbreviations of species are given in Table 2.

Position of nucleotide	**TRAE**	**TRDI**	**TRMB**	**TRUR**
1028.	C	C	A	A
1323.	C	C	T	T
Position of protein				
343.	C**C**A (proline)	C**C**A (proline)	C**A**A (glutamine)	C**A**A (glutamine)
441.	TG**C** (cysteine)	TG**C** (cysteine)	TG**T** (cysteine)	TG**T** (cysteine)

Recent studies have shown that *mat*K sequence analyses provide useful information about phylogenie and genetic distance, particularly above the genus level (10, 15,16). We selected 16 individiuals which have high polimorphism level according to AFLP analysis which we studied earlier (unpublished data). However, we did not find enough polymorphism for a phylogenetic interpretation. It is not enough to make a phylogenetical interpretation by using the data based only on two base changes. Although *mat*K gene provides phlogenetically informative data at species and up to species level in general, it has been found phlogenetically noninformative for the species studied.

We did not find any correlation between the genetic distance and ecological and climatical factors in this study.

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CHARACTERIZATION OF BETA-GALACTOSIDASE FROM *Alternaria alternata*

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Abstract

In this study, optimum conditions affecting the activity of beta-galactosidase enzyme from *Alternaria alternata* were determined and its stability was investigated. It was concluded that 0.75% ONPG concentration is suitable for high beta-galactosidase activity. The optimum temperature and pH were found to be 50°C and 5, respectively. The enzyme was stable between 20-50°C in the pH range 3.0-7.0 and kept more than 50% of its original activity after 40 min at 60°C.

Key Words: beta-galactosidase, lactase, stability, enzyme

Introduction

Lactose is the main carbohydrate contained in milk and whey [1]. The consumption of foods with a high content of lactose causes problem for the majority of the world population, known as lactose intolerance [2]. Therefore, in order to avoid this problem lactose should be broken into simpler forms such as glucose and galactose. On the other hand, whey is an abundant effluent produced in cheese and casein manufacture. The biotechnological utilization of this economically valuable feedstock is largely limited by lactose due to its poor solubility and insufficient sweetness [3]. Beta-galactosidase enzyme is needed for eliminating these disadvantages and making use of the lactose content of whey [4].

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Due to the above-mentioned facts, hydrolysis of lactose with microbial beta-galactosidase (beta-D-galactoside galactohydrolase, E.C.3.2.1.23) has found commercial use in the food industry for the preparation of low-lactose dairy products for people suffering lactose intolerance as well as for the preparation of new foods and dairy products [5]. For example, in dairy industry beta-galactosidases are widely used in the production of ice cream and sweetened flavoured and condensed milks. Yeast beta-galactosidase, with an optimum pH in the range of 6.0-7.0, is quite adequate for hydrolyzing milk [5]. Beta-galactosidases from various fungi (mould) with pH acid optima are more suitable to whey hydrolysis [6].

In most of the previous studies found in literature, much research was carried out to determine the pH and temperature ranges in which the enzymes are stable as well as the kinetic studies performed in a wide range of temperatures and substrate concentrations [7]. In this respect, in the scope of this study optimum conditions affecting the activity of beta-galactosidase enzyme from *Alternaria alternata* were determined and its stability was investigated.

Materials and Methods

Microorganism: In the study, *Alternaria alternata* strain in Hacettepe University Microbiology Laboratory was used for the production of beta-galactosidase enzyme. Stock cultures were maintained on potato dextrose agar at 4°C.

Medium: Medium described by Fiedurek and Ilczuk [8] was used with some modification for growth and enzyme production. Medium contains (as gl^{-1}); 10.0 lactose, 1.5 peptone, 1.0 yeast-extract, 1.0 KH_2PO_4, 7.0 $(NH_4)H_2PO_4$, 1.0 $MgSO_47H_2O$ and 0.3 $CaCl_2$. In 250 ml flasks, 50 ml of media with pH 5 were sterilized in autoclave at 121°C, 1.5 atm for 15 min.

Inoculation: Spore suspensions containing 3.10^6 spores/ml was inoculated into 50 ml growth media.

Determination of enzyme activity: Beta-galactosidase activity was determined with the method described by Reczey *et al.* [9]. The culture was centrifuged at 7,200 rpm for 15 min and used as the enzyme sample. Enzyme activations were determined as follows:

1. Enzyme was assayed with 2.5 mg/ml o-nitrophenyl-β-D-galactopyranoside (ONPG) as substrate (Merck), which was prepared in 0.1 M sodium-acetate buffer with pH 5.
2. One ml of substrate solution and 0.2 ml enzyme sample were added into reaction tubes.
3. The mixture was incubated at 50°C for 15 min.
4. Reaction was terminated by adding one ml of 10% sodium carbonate into the reaction tube.

5. The absorbance was read at 420 nm using Jenway, 6105 u.v., vis spectrophotometer. The amount of o-nitrophenol was calculated from the standard curve.

One unit of beta-galactosidase activity was described as the amount of enzyme producing one μmole of o-nitrophenol in one ml medium at 50°C in one minute.

Determination of the properties of beta-galactosidase enzyme: The medium was incubated at 150 rpm for 6 days at 30°C and supernatant of *A.alternata* was used as the enzyme source. In order to determine the effect of temperature on enzyme activity, the medium was incubated at different temperatures between 20-60°C to determine the enzyme activities. Similarly, in order to determine the optimum pH value, the medium was incubated at different pH values between 4.0-6.0. Enzyme activities were determined as relative activity. In addition, enzyme activities of different ONPG concentrations between 0.25-3.00% (in sodium acetate buffer) were determined after incubation at 50°C for 15 min.

Temperature and pH stability of the enzyme: Enzyme stabilities at different temperatures between 20-50°C were determined after incubation for one hour. Next, in order to determine the stability of the enzyme at 60°C, enzyme activities were measured at 20, 30 and 40 min. In addition, enzyme stabilities between pH 3.0-7.0 were determined after incubation. In this respect, enzyme activities were determined as relative activity.

Results and Discussion

First of all, effect of incubation temperature on beta-galactosidase activity was determined. Experimental results showed that beta-galactosidase activity increases up to 50°C and is maximum around this temperature (Figure 1). Therefore, it was concluded that optimum temperature for maximum beta-galactosidase enzyme activity from *A.alternata* was 50°C. This can be considered as an advantage of this enzyme especially when used in commercial applications.

Secondly, results of the experiments carried out at different pH values reveal that the activity was maximum at pH 5 (Figure 2), which is parallel with the findings of previous studies found in literature [10,11]. In similar studies carried out with fungal sources, optimum temperatures and pH values were found around 50°C and in 4.0-5.0 range, respectively [8,10,12,13].

When the effect of substrate concentration on beta-galactosidase activity was studied, it was concluded that the optimum ONPG concentration was 0.75% as the activity increases up to this point and remains constant at higher concentrations (Figure 3).

For industrial enzymes, temperature and pH stability is of great importance. In this respect, temperature stability of the produced beta-galactosidase enzyme was studied and the results showed that the enzyme kept more than 50% of its original activity between 20-50°C (Figure 4). Although in the first part of this study optimum temperature was found to be 50°C, it can be seen from Figure 4 that enzyme activity decreases above 40°C for longer incubation periods, which is probably due to the fact that at high temperatures structure of the enzyme changes. In addition, when the activity of beta-galactosidase enzyme at 60°C was studied, it was observed that the enzyme kept 53% of its original activity after 40 min (Figure 5).

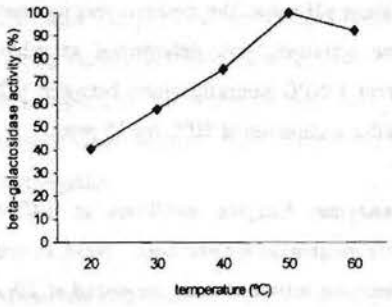

Figure 1. Effect of temperature on beta-galactosidase activity

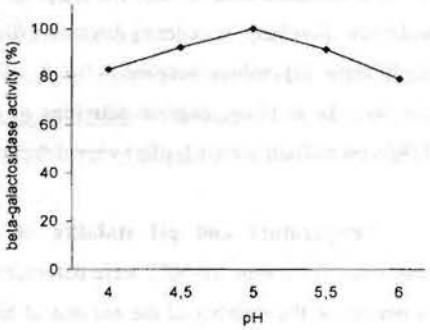

Figure 2. Effect of pH on beta-galactosidase activity

Similar to our findings, in a previous study on beta-galactosidase stability, the enzyme produced from *P.notatum* was high between 20-40°C and decreased above 50°C after incubation for one hour [8]. In another study carried out with *B.bassiana* optimum temperature was found to be 50°C, whereas the activity reduced to 5.9% at 60°C after 30 min [14].

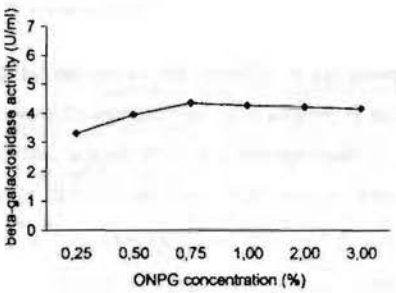

Figure 3. Effect of ONPG concentration on beta-galactosidase activity

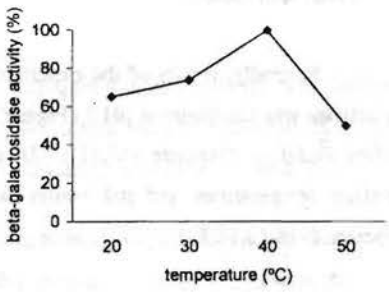

Figure 4. Temperature stability of the enzyme

When pH stability of the enzyme was investigated it was concluded that between pH values 3.0-7.0 enzyme activity was above 90% after one hour, which implies that this enzyme is highly stable from pH point of view (Figure 6).

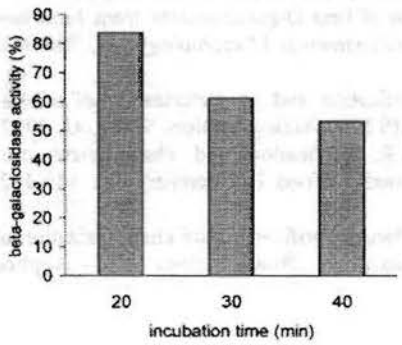

Figure 5. Beta-galactosidase activity at 60°C

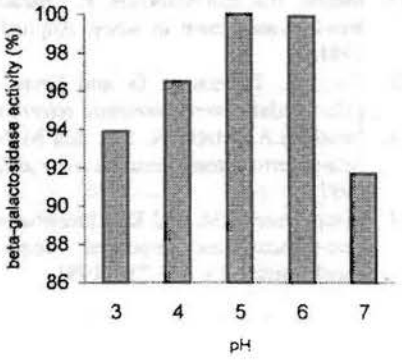

Figure 6. pH stability of the enzyme

As a result, it was concluded that 0.75% ONPG concentration is suitable for high beta-galactosidase activity. The optimum temperature and pH were found to be 50°C and 5.0, respectively. The enzyme was stable between 20-50°C in the pH range 3.0-7.0 and kept more than 50% of its original activity after 40 min at 60°C.

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STUDIES ON THE SYNTHESIS OF TETRACYCLIC INDOLE ALKALOIDS. SYNTHESIS OF AZOCINO[4,3-b]INDOLE FRAMEWORK

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Abstract

The synthesis of the compound **10** which has hexahydro-1,5-methanoazocino[4,3-b]indole structure for the synthesis of pentacyclic strychnos type of alkaloids (tubifolin and tubifolidine) was described. Many new tetrahydrocarbazol intermediates and hexahydro-1,5-methanoazocino[4,3-b]indole derivative (5, 6a, 6b, 6c, 6d, 7, 8,10) have also been synthesized. The structures were confirmed by NMR, MS, FT-IR techniques and elemental analysis.

Key words: Alkaloids, indole alkaloids, strychnos alkaloids, azocino[4,3-b]indole.

Introduction

Several routes to tetracyclic substructures (1) of the strychnos-type alkaloids have been reported in the literature (1-7). Most of them start with the aromatic A-ring and the heterocyclic D-ring and build up the tetracyclic skeleton by closing the rings B and C later.

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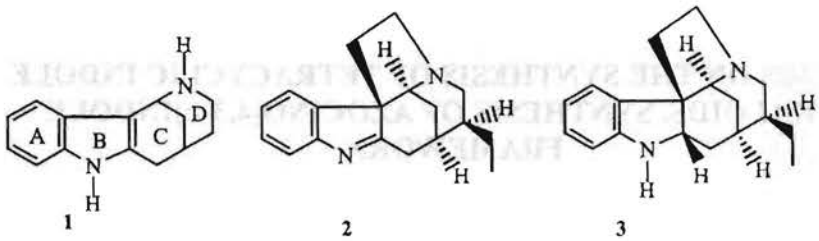
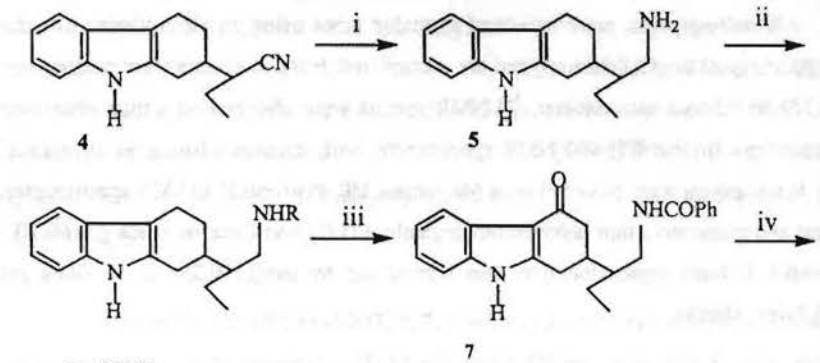
In this paper we describe a synthetic route utilizing tetrahydrocarbazole which bears an amide side chain at the position 2 (carbazole numbering) as a key-intermediate. This carbazole-derivative allows the closure of the D-ring in the last synthetic step, yielding a tetracyclic framework for many indole alkaloids. The scope of this approach is valuable for the construction of the more highly substitued alkaloids tubifolin (**2**) and tubifolidine (**3**) (8-10).



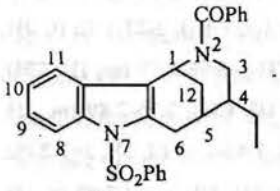
1 **2** **3**

In this study we synthcsized tetracyclic structure (10) which has hexahydro-1,5-methanoazocino [4,3-b]indole structure for the synthesis of pentacyclic strychnos alkaloids (tubifolin and tubifolidin). For the preparation of the open chain precursor **8** we developed a simple route using mild reaction conditions and easily available starting materials. Proceeding from 2-(l, 2, 3, 4-tetrahydrocarbazol-2-yl)-butyronitril (**4**) the amine **5** is formed by reduction with lithium aluminium hydride (11,12). Attempts to obtain homologous amides **6a, 6b, 6c** and **6d** under standard conditions were carried out with related acyl halide and anhydride (13). The only succesful oxidation of **6d** with DDQ afforded 4-oxo-l, 2, 3, 4-tetrahydrocarbazole **7** (14-16). In the next step the indole N-H of **7** was protected with benzenesulfonyl chloride using the phase- transfer technique (17). The cyclization constitutes the application of a known process of ring closure (7). This approach implies a further reduction step of **8** with sodium boronhydride in order to generate the 4-hydroxy-1, 2, 3, 4-tetrahydrocarbazole **9** moiety for closure of the ring. The ring closure was achieved by treatment with trifloroacetic acid to yield a tetracyclic skeleton which constitutes the skeletal framework of the hexahydro-1,5-methanoazocino [4,3-b]indole **10**.

Scheme1



6a: $COCF_3$
6b: $COOC(CH_3)_3$
6c: $COCH_3$
6d: COPh



Reagents and Conditions: i) $LiAlH_4$, THF, reflux, 5h, 88%; ii) for **6a**, CF_3CO_2H, $ClCO_2C_2H_5$, $(C_2H_5)_3N$, stirred at 0°C, 1h, then compound **5**, 25°C, 12h, 17%; for **6b**, ditertbutyl dicarbonate, stirred at room temperature, 1h, 88%; for **6c**, $(C_2H_5)_3N$, Ac_2O, stirred at room temperature, 1h, 85%; for **6d**, $(C_2H_5)_3N$, PhCOCl, stirred at room temperature, 1h, 73%; iii) DDQ, THF (90%), stirred, 5h, 48%; iv) NaOH, TBAHS, $PhSO_2Cl$, stirred, 1h, 91%; v) $NaBH_4$, CH_3OH-THF, stirred, 2h; vi) CF_3CO_2H, stirred, 12h, 57%.

Experimental

All melting points were measured in sealed tubes using an electrothermal digital melting point apparatus (Gallenkamp) and are uncorrected. Infrared spectra were recorded on Hitachi 270-30 infrared spectrometer. ^{1}H NMR spectra were obtained on a high resolution fourier transform Bruker WH-400 NMR spectrometer with tetramethylsilane as an internal stantard. Mass spectra were recorded on a Micromass UK Platform II LC-MS spectrometer. Analytical and preparative thin layer chromatography (TLC) was done on silica gel 60 HF-254 (Merck). Column chromatography was carried out by using 70-230 mesh silica gel (0.063-0.2 mm, Merck).

2-(1, 2, 3, 4-Tetrahydrocarbazol-2-yl)-butylamine (5). To a solution of **4** (5 g, 21 mmoles) in tetrahydrofuran (50 mL) was added lithium aluminium hydride (2.40 g, 62 mmoles) slowly at 0°C. The reaction mixture was refluxed 5 h under nitrogen, then excess of lithium aluminium hydride was destroyed with cold water. The reaction mixture was extracted with ethyl acetate. The organic layer was dried with anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The residue was chromatographed using silica gel and ethyl acetate- methanol (9:1) to afford **5** (4.5 g, 88%) as a colorless solid, mp: 152-153°C. TLC: Rf 0.12 (ethyl acetate-methanol 9:1). IR (KBr) cm^{-1}: 3400 (NH), 3250 (NH), 2980 (CH). ^{1}H NMR ($CDCl_3$) δ: 0.95-1.05 (t, 3H, J=7.2 Hz, CH_3), 1.22-1.26 (t, 2H, J=7.1 Hz, NH_2), 1.37-1.45 (m, 4H, 2xCH_2), 1.53-1.64 (m, 2H, CH_2), 1.98-2.07 (m, 1H, CH), 2.09-2.12 (m, 1H, CH), 2.56-2.65 (m, 1H, CH), 2.67-2.72 (m, 1H, CH), 2.76-2.89 (m, 2H, CH_2), 7.07-7.15 (m, 2H, ArH), 7.26-7.30 (d, 1H, J=7.4 Hz, ArH), 7.45-7.49 (d, 1H, J=7.2 Hz, ArH), 7.82 (s, 1H, NH). ^{1}H NMR ($CDCl_3$, D_2O) δ: 1.22-1.26 (t, 2H, NH_2) and 7.82 (s, 1H, NH) signals disappeared. MS m/z (rel.int.): 243 (11.6) $[M+1]^+$, 242 (55) $[M]^+$, 225 (16.5) $[M-NH_3]^+$, 212 (17) $[M-C_2H_6]^+$, 170 (73) $[M-C_4H_{10}N]^+$, 115 (33) $[M-C_8H_{17}N]^+$. Anal. Calcd. for $C_{16}H_{22}N_2$: C, 79.34; H, 9.09; N, 11.57. Found: C, 79.30; H, 9.11; N, 11.59.

N-Trifloroacetyl-2-(1, 2, 3, 4-tetrahydrocarbazol-2-yl)-butylamine (6a). A solution of trifloroacetic acid (2 g, 17.54 mmoles) was added dropwise to a solution of ethyl chloroformate (2 g, 18.43 mmoles) in dichloromethane (5 mL) at 0°C followed by triethyl amine in dichloromethane. The solution was stirred 1 h at 0°C and compound **5** (4.84 g, 17.54 mmoles) was added. After 12 h at 25°C the reaction mixture was diluted with ethyl acetate (100 mL) and washed with saturated sodium chloride. The organic layer was dried with anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The crude product was chromatographed using silica gel and ethyl acetate to give **6a** (1.05 g,

17%), mp: 225-226°C. TLC: Rf 0.67 (ethyl acetate). IR (KBr) cm^{-1}: 3400 (NH), 3300 (NH), 2940 (CH), 1710 (amide C=O). ^1H NMR ($CDCl_3$) δ: 0.98-1.02 (t, 3H, J=7.3 Hz, CH_3), 1.21-1.34 (m, 4H, 2xCH_2), 2.01-2.04 (m, 2H, CH_2), 2.57- 2.66 (m, 3H, CH and CH_2), 2.82-2.86 (m, 1H, CH), 3.29-3.51 (m, 2H, CH_2), 4.22 (s, 1H, NH), 7.07-7.14 (m, 2H, ArH), 7.25 (d, 1H, J=7.2 Hz, ArH), 7.45 (d, 1H, J=7.3 Hz, ArH), 7.58 (s, 1H, NH). MS m/z (rel.int.): 269 (1.5) $[M-CF_3]^+$, 212 (1.5) $[M-C_3H_3NOF_3]^+$, 170 (12.40) $[M-C_6H_9NOF_3]^+$, 115 (12.76) $[M-C_{10}H_{16}NOF_3]^+$. Anal. Calcd. for $C_{18}H_{21}N_2OF_3$: C, 63.90; H, 6.21; N, 8.28. Found: C, 63.85; H, 6.24; N, 8.25.

N-tert-Butoxycarbonyl-2-(1, 2, 3, 4-tetrahydrocarbazol-2-yl)butylamine (6b). A solution of **5** (2 g, 8.26 mmoles) in dichloromethane (10 mL) was cooled to 0°C and then di-tert-butyl dicarbonate (2 g, 9.16 mmoles) was added and stirred 1 h at room temperature. The reaction mixture was diluted with water and extracted with chloroform. The organic layer was first washed with hydrochloric acid (50 mL, 10%) and then sodium carbonate (50 mL, 10%). The organic layer was dried with anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The residue was chromatographed using silica gel and ethyl acetate. After the solvent was evaporated recrystallization from methanol yielded **6b** (2.5 g, 88%), mp: 160-161°C. TLC: Rf 0.71 (ethyl acetate). IR (KBr) cm^{-1}: 3400 (NH), 3350 (NH), 2980 (CH), 1675 (amide C=O). ^1H NMR ($CDCl_3$) δ: 0.90 (t, 3H, J=7.4 Hz, CH_3), 1.25-1.35 (m, 4H, 2xCH_2), 1.40-1.45 (s, 9H, 3xCH_3), 1.89-1.91 (m, 2H, CH_2), 2.45-2.65 (m, 3H, CH and CH_2), 2.73-2.76 (m, 1H, CH), 3.10-3.25 (m, 2H, CH_2), 4.50 (s, 1H, NH), 6.97-7.07 (m, 2H, ArH), 7.20 (d, 1H, J=7.8 Hz, ArH), 7.37 (d, 1H, J=7.4 Hz, ArH), 7.60 (s, 1H, NH). MS m/z (rel.int.): 343 (9.80) $[M+1]^+$, 342 (38) $[M]^+$, 285 (29) $[M-C_4H_9]^+$, 241 (8) $[M-C_5H_9O_2]^+$, 170 (100) $[M-C_9H_{18}NO_2]^+$. Anal. Calcd. for $C_{21}H_{30}N_2O_2$; C, 73.68; H, 8.77; N, 8.19. Found: C, 73.71; H, 8.71; N, 8.23.

N-Acetyl-2-(l, 2, 3, 4-tetrahydrocarbazol-2-yl)-butylamine (6c). A solution of **5** (2 g, 8.26 mmoles) in dichloromethane (20 mL) was cooled to 0°C and then triethyl amine (2.5 g, 27.45 mmoles) was added. Acetic anhydride (1 g, 9.80 mmoles) was added slowly and stirred 1 h at room temperature. The reaction mixture was diluted with water and extracted with chloroform. The organic layer was first washed with hydrochloric acid (50 mL, 10%) and then sodium carbonate (50 mL, 10%). The organic layer was dried with anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The residue was chromatographed using silica gel and ethyl acetate. After the solvent was evaporated recrystallization from methanol yielded **6c** (2 g, 85%), mp: 85-86°C. TLC: Rf 0.61 (ethyl acetate). IR (KBr) cm^{-1}: 3450 (NH), 3300 (NH), 2980 (CH), 1660 (amide C=O). ^1H NMR ($CDCl_3$) δ: 0.90 (t, 3H, J=7.2 Hz, CH_3), 1.20-1.60 (m, 4H, 2xCH_2), 1.89 (s, 3H, $COCH_3$),

1.92-1.98(m, 2H, CH_2), 2.50-2.65 (m, 3H, CH and CH_2), 2.75-2.85 (m, 1H, CH), 3.15-3.45 (m, 2H, CH_2), 5.35 (s, 1H, NH), 6.98-7.09 (m, 2H, ArH), 7.21 d, 1H, J=7.8 Hz, ArH), 7.37 (d, 1H, J=7.4 Hz, ArH), 7.65 (s, 1H, NH). MS m/z (rel.int.): 285 (7) $[M+1]^+$, 284 (48) $[M]^+$, 226 (2) $[M-C_2H_4NO]^+$, 170 (100) $[M-C_6H_{12}NO]^+$. Anal. Calcd. for $C_{18}H_{24}N_2O$: C, 76.06; H, 8.45; N, 9.86. Found: C, 76.11; H, 8.39; N, 9.84.

N-Benzoyl-2-(1, 2, 3, 4-tetrahydrocarbazol-2-yl)-butylamine (6d). A solution of **5** (2 g, 8.26 mmoles) in dichloromethane (20 mL) was cooled to 0°C and then triethyl amine (2.5 g, 27.45 mmoles) was added. Benzoyl chloride (1.2 g, 8.54 mmoles) was added slowly and stirred 1 h at room temperature. The reaction mixture was diluted with water and extracted with chloroform. The organic layer was first washed with hydrochloric acid (50 mL,10%) and then sodium carbonate (50 mL,10%). The organic layer was dried with anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The residue was chromatographed using silica gel and ethyl acetate. After the solvent was evaporated recrystallization from methanol yielded **6d** (2.10 g, 73%), mp: 88-89°C. TLC: Rf 0.63 (ethyl acetate). IR (KBr) cm^{-1}: 3350 (NH), 3250 (NH), 2980 (CH), 1650 (amide C=O). ^1H NMR ($CDCl_3$) δ: 0.98 (t, 3H, J=7.3 Hz, CH_3), 1.50-1.63 (m, 2H, CH_2), 1.76-1.79 (m, 2H, CH_2), 1.97-2.09 (m, 2H, CH_2), 2.55-2.82 (m, 4H, 2xCH and CH_2), 3.50-3.69 (m, 2H, CH_2), 6.05 (s, 1H, NH), 6.95-7.05 (m, 2H, ArH), 7.25-7.45 (m, 5H, ArH), 7.60-7.70 (m, 2H, ArH), 8.01 (s, 1H, NH). MS m/z (rel.int.): 346 (12) $[M]^+$, 241 (3) $[M-C_7H_5O]^+$, 170 (100) $[M-C_{11}H_{14}NO]^+$. Anal. Calcd. for $C_{23}H_{26}N_2O$: C, 79.77; H, 7.51; N, 8.09. Found: C, 79.74; H, 7.46; N, 8.11.

N-Benzoyl-2-(1, 2, 3, 4-tetrahydrocarbazol-4-one-2-yl-butylamine (7). 2,3-dichloro-5,6- dicyano-p-benzoquinone (DDQ, 3.28 g, 14.45 mmoles) in tetrahydrofuran (10 mL) was added dropwise to a solution of **6d** (2.5 g, 7.23 mmoles) in tetrahydrofuran (20 mL, 90%) at 0°C. The reaction mixture was stirred 5 h at room temperature, then the solution was poured into sodium hydroxide (500 mL, 10%) and extracted with ethyl acetate. The organic layer was dried with anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The residue was purified by chromatography using silica gel and ethyl acetate-benzene (1:1) to afford **7** (1.25 g, 48%), mp: 213-214°C. TLC: Rf 0.25 (ethyl acetate-benzene l: l). IR (KBr) cm^{-1}: 3300 (NH), 3200 (NH), 2980 (CH), 1680 (ketone C=O), 1650 (amide C=O). ^1H NMR (d_6- DMSO)δ: 1.04 (t, 3H, J=7.2 Hz, CH_3), 1.38-1.41 (m, 1H, CH), 1.52-1.62 (m, 1H, CH), 2.41-2.56 (m, 3H, CH and CH_2), 2.83-2.89 (m, 3H, CH and CH_2), 3.36-3.41 (m, 1H, CH), 3.48-3.54 (m, 1H, CH), 6.50 (s, 1H, NH), 7.11- 7.13 (d, 1H, J=7.2 Hz, ArH), 7.30-7.46 (m, 4H, ArH), 7.80-7.86 (m, 2H, ArH), 8.01 (m, lH, ArH), 8.23 (m, 1H,

ArH), 11.52 (s, 1H, NH). MS m/z (rel.int.): 361 (0.4) $[M+1]^+$, 360 (0.5) $[M]^+$, 184 (5.50) $[M-C_{11}H_{14}NO]^+$. Anal. Calcd. for $C_{23}H_{24}N_2O_2$: C, 76.67; H, 6.67; N, 7.77. Found: C, 76.70; H, 6.69; N, 7.74.

N-Benzoyl-2-(1,2,3,4-tetrahydrocarbazol-9-benzenesulfonyl-4-one-2-yl)-butyl amine (8). A solution of **7** (1.50 g, 4.17 mmoles) in dichloromethane (50 mL) was cooled to 0°C. Solution of sodium hydroxide (5 mL, 50%), 100 mg of tetrabutylammonium hydrogen sulfate and benzene sulfonyl chloride (0.76 g, 4.30 mmoles) were added and stirred 1 h at room temperature. After 1 hour reaction mixture was washed with hydrochloric acid (50 mL, 10%) and the organic layer was dried with anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure and the resulting residue was chromatographed using silica gel and ethyl acetate to afford **8** (1.90 g, 91%), mp: 103-104°C. TLC: Rf 0.58 (ethyl acetate). IR (KBr) cm^{-1}: 3300 (NH), 2980 (CH), 1690 (ketone C=O), 1670 (amide C=O), 1350 and 1170 (S=O). ^1H NMR ($CDCl_3$) δ: 1.05 (t, 3H, J=7.45 Hz, CH_3), 1.30-1.45 (m, 2H, CH_2), 1.70-1.82 (m, 2H, CH_2), 1.87-1.96 (m, 1H, CH), 2.17-2.25 (m, 2H, CH_2), 2.33-2.37 (m, 1H, CH), 2.90-2.96 (dd, 1H, J=13.54 and 3.52 Hz, CH), 3.48-3.53 (dd, 1H, J=13.89 and 5.31 Hz, CH), 5.45 (t, 1H, J=2.80 Hz, NH), 7.05-7.12 (m, 2H, ArH), 7.18-7.36 (m, 4H, ArH), 7.44-7.49 (m, 3H, ArH), 7.52-7.58 (m, 2H, ArH), 7.82-7.90 (m, 3H, ArH). MS m/z (rel.int.): 501 (4) $[M+1]^+$, 500 (6) $[M]^+$, 359 (1.5) $[M-C_6H_5SO_2]^+$, 254 (1.5) $[M-C_{13}H_{10}SO_3]^+$. Anal. Calcd. for $C_{29}H_{28}N_2SO_4$: C, 69.60; H, 5.60; N, 5.60. Found: C, 69.52; H, 5.62; N, 5.64.

2-Benzoyl-7-benzenesulfonyl-4-ethyl-1,2,3,4,5,6-hexahydro-1,5-methanoazocino [4,3-b]indole (10). A solution of **8** (1 g, 2 mmoles) in methanol-tetrahydrofuran (10mL, 1:1) was cooled to 0°C and treated with sodium borohydride (0.150 mg, 4 mmoles) and the mixture stirred for 2 h at room temperature. The reaction mixture was diluted with water and extracted with chloroform. The organic layer was dried with anhydrous magnesium sulfate. The solvent was evaporated to yield oily crude product **9** (0.90g). Compound **9** (1 g, 1.99 mmoles) was dissolved in dichloromethane (15 mL) and cooled to 0°C. The reaction mixture was treated with trifloroacetic acid (0.25 g, 2.19 mmoles) and stirred for 12 h at room temperature. After 12 h the reaction mixture was poured into water and extracted with dichloromethane. The organic layer was dried with anhydrous magnesium sulfate and the solvent was evaporated. The crude product was chromatographed with silica gel and ethyl acetate. The solvent was removed under reduced pressure and the product was crystallized from methanol to afford **10** (0.55 g, 57%), mp: 135- 136°C. TLC: Rf 0.51 (ethyl acetate). IR (KBr) cm^{-1}: 2980 (CH), 1680 (amide C=O), 1350 and 1170 (S=O). ^1H NMR ($CDCl_3$) δ: 0.95 (t, 3H, J=7.30 Hz, CH_3),1.30-1.42 (m, 1H, CH), 1.46-1.62 (m, 1H, CH), 1.67-1.75 (m, 1H, CH), 1.82-2.10 (m, 2H, CH_2), 2.47-2.70 (m, 2H, CH_2), 2.75-2.87 (m, 1H, CH),

3.05-3.10 (m, 2H, CH_2), 4.85 (t, 1H, J=6.14 Hz, 1-CH), 7.08-7.17 (m, 2H, ArH), 7.25-7.30 (m, 2H, ArH), 7.43-7.63 (m, 6H, ArH), 7.68-7.73 (m, 1H, ArH), 7.87-7.94 (m, 3H, ArH). MS m/z (rel.int.): 485 (6) $[M+1]^+$, 484 (12) $[M]^+$, 343 (29) $[M-C_6H_5SO_2]^+$, 238 (8) $[M-C_{13}H_{10}SO_3]^+$, 168 (12.5) $[M-C_{17}H_{18}NSO_3]^+$. Anal. Calcd. for $C_{29}H_{28}N_2SO_3$: C, 71.90; H, 5.78; N, 5.78. Found: C, 71.82; H, 5.82; N, 5.81.

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