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Estimation of Adult Density of *Aedes albopictus* (Diptera: Culicidae) in Some Hilly Areas of Pakistan

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Abstract.- *Aedes albopictus* is a vector of dengue and chikungunya. In present study we recorded the adult density of *A. albopictus* in four different cities in Pakistan (Rawalpindi, Islamabad, Abbotabad and Mansehra). The highest adult density of *A. albopictus* was observed at Shakarparian (Rawalpindi). We found a negative correlation between density of *A. albopictus* and elevation. However, there was a strong positive correlation between adult densities of *A. albopictus* and human population, vegetation thickness, temperature and rain fall. Present study is the first report on *A. albopictus* density in the study areas. This study will be helpful for mosquito researchers and public health professionals and agencies to understand the ecology and distribution of *A. albopictus* and aid in developing better strategies for the control of this mosquito species in the above mentioned study areas.

Keywords: *Aedes albopictus*, dengue, adult density, dengue fever

For the last few years there is an unprecedented increase in dengue fever epidemic in Pakistan, especially in the post monsoon period (Sherin, 2011; Shakoora *et al.*, 2012). There are two major mosquito species that contribute to dengue outbreak in this region, *Aedes aegypti* (Linnaeus) and *A. albopictus* (Skuse) (Jeefoo *et al.*, 2010). *A. albopictus* is a vector of more than 20 arboviruses (Benedict *et al.*, 2007; Paupy *et al.*, 2011; Delatte *et al.*, 2013) and is native to Asia (Skuse, 1894). But now this mosquito is rapidly expanding its global range and importance in transmitting dengue viruses (Gratz, 2004; Nelder *et al.*, 2010). *A. albopictus* shows high plasticity in adoption of habitat (Hafeez and Akram, 2011).

A. albopictus bites during the day time (Benedict *et al.*, 2007) and is regarded as less important vector in transmitting dengue because it also feeds on animals other than humans contrary to the highly anthropophilic *A. aegypti* (Hawley, 1988; Reiter *et al.*, 2006). Higher densities of *A. albopictus* are found in the peridomestic environment, particularly in areas with plentiful vegetation (Reiter, 2010). But according to Phillips (2008), *A. albopictus* is an important dengue vector in rural and suburban areas in Southeast Asia. *A. albopictus* is also found to be highly aggressive to humans and might therefore be involved in human-human virus transmission (Kamgang *et al.*, 2012).

In the present study we recorded the population density of *A. albopictus* in Rawalpindi, Islamabad, Abbotabad and Mansehra of Pakistan. We were also interested in finding out the maximum vertical flight range of *A. albopictus*, correlation of adult density of it with elevation and population density of humans, vegetation thickness, temperature and rainfall. During review of literature, we did not find any published data on the population estimation of *A. albopictus* in the above-mentioned areas. Therefore, this study will be the first report on *A. albopictus* density in above mentioned areas. This study will be helpful in devising strategies for the prevention and control of *A. albopictus* in these areas.

Methodology

The adult densities of *A. albopictus* was recorded July - September 2012 in Rawalpindi, Islamabad, Abbotabad and Mansehra, Pakistan (Fig.1). Information regarding the locality of sites,

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longitude, latitude, elevation, rainfall, temperature and vegetation thickness was also obtained. To record the adult density of *A. albopictus* one member of our team stayed at each study site for 45 minutes from his body during were collected with the help of aspirator. The mosquitoes were then identified to confirm the species. We categorized the study sites on the basis of number of mosquitoes recorded in 45 minutes as follows: i) very high density site (≥ 150); ii) high density site (100-149); iii) average density site (99 ≤ but ≥ 50); iv) low density site (49 ≤ but ≥ 10); v) very low density site (9 ≤ but ≥ 1) and vi) zero density site (no mosquito)

Pearson's correlation was then used to find out the relationship of density of *A. albopictus* with elevation, human population at each study site, vegetation thickness, temperature and rainfall. Statistical analyses were performed using SPSS (Ver. 16).

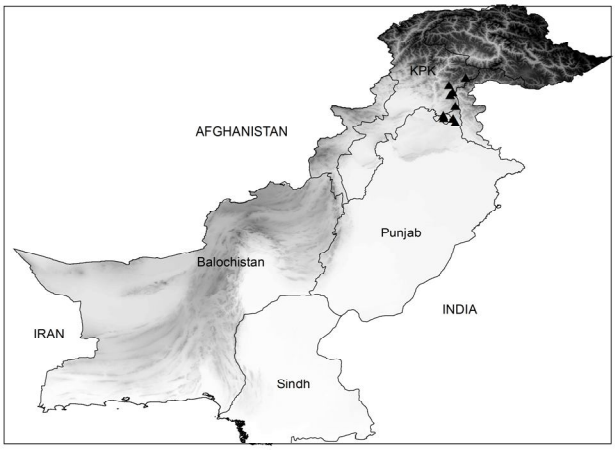

Fig. 1. Map showing data collection sites.

Results

Detail of the collected information about locality of sites, longitude, latitude and elevation as well as categories of sites based on the population density of *A. albopictus* is depicted in the Table I. The highest density of *A. albopictus* was recorded at Shakarparian (Rawalpindi). There was a negative correlation between density of *A. albopictus* and elevation (Pearson correlation = -0.431; P = 0.214). However the density of *A. albopictus* was positively correlated with human population (Pearson correlation = 0.91; P<0.001), vegetation thickness (Pearson correlation = 0.881; P < 0.001), temperature (Pearson correlation = 0.79; P < 0.001) and rainfall (Pearson correlation = 0.95; P < 0.001).

Discussion

A. albopictus is generally considered as an inefficient vector of dengue as it is not well adapted to urban domestic environments and is less anthropophilic than *A. aegypti* (Rezza, 2012). Furthermore, feeding of this mosquito on multiple animals especially mammals decreases the probability of its feeding on humans (Rodhain and Rosen, 1997; Reiter *et al.*, 2006). In our study we found higher densities of *A. albopictus* at Shakarparian and Margalla National Park, both of which are public recreational spots and have lower number of other mammals. So visitors to these places might be at a higher risk of *A. albopictus* bites in the absence of alternative hosts for feeding.

In many parts of the world *A. albopictus* is competing with *A. aegypti* resulting in rapid decline of *A. aegypti* density. This shift is particularly important from public health perspective as *A. albopictus* is less efficient in transmitting dengue virus (Kaplan *et al.*, 2010). During our three months collecting period, we were not able to collect even a single *A. aegypti*. But we can not exclude the possibility of the presence of *A. aegypti* in densely populated areas since we collect only from forests and vegetations near the public places and near scattered residential areas, which are not the preferred habitat of *A. aegypti.*

As we moved vertically on the mountainous areas we observed a regular drop in the temperature and mosquito density. We did not find any *A. albopictus* above 3650 feet. Watson (1967) had quoted some references in his review article about the presence of *A. albopictus* in the forest at the altitude of 2000 feet or higher. According to Alto and Juliano (2001), populations of *A. albopictus* in the regions with relatively higher summer temperatures are likely to have high rates of population growth with populations of adults peaking early in the season.

Khan *et al*. (2011) reported in their study that they collected *A. albopictus* from mountains of

Table I.- Detail of study sites and their categories based on density of *Aedes albopictus*.

District	Tehsil	Locality	Latitude	Longitude	Elevation (ft)	Category based on mosquito density
Islamabad	Islamabad	Margalla National Park, 2.5 Km N Damen-e-Koh to Monal Road	33°44.865'N	73°03.452'E	3179	high density site
Islamabad	Pir Sohawa	17 kilometers from Islamabad on top of "Margalla Hills"	33°45.37'N	73°03.562'E	3650	low density site
Rawalpindi	Kahuta	Sumbla hills Kahuta, 10 km W to Lethrar on Kahuta to Lethrar road	33°38.540'N	73°23.822'E	2394	very low density site
Rawalpindi	Murree	14 km Lethrar to Neloir (on Lethrar road), 800 m W from lethrar road.	33°40.012'N	73°20.182'E	2057	very low density site
Rawalpindi	Murree	Kuldana, 2 km N from Murree to Ayubia	34°39.150'N	73°13.40'E	7240	zero density site
Abbotabad	Abbotabad	Ayubia National Park, 3 km S from Ayubia to Dunga Gali track	34°02.266'N	73°24.039'E	7684	zero density site
Mansehra	Mansehra	8 km N Mansehra to Naran road toward Naran	34°21.721'N	73°15.535'E	3421	zero density site
Mansehra	Balakot	Damgala, 25 km N Mansehra to Naran road toward Naran	34°27.042'N	73°19.883'E	3650	very low density site
Mansehra	Balakot	Near Lake Saif- Ul- Malook, 9 km E from Naran	34°52.864'N	73°41.746'E	10600	zero density site

Islamabad in 2005 but the information regarding exact locality, longitude, latitude and elevation is missing. In our study, we also noted that *A. albopictus* prefers thick vegetated and shady areas. Chen *et al*. (2006) and Khan and Sulman (1969) have also reported significant association of densities of *A. albopictus* with thick vegetation.

Higher densities of *A. albopictus* were recorded in the 3rd week of August at the collection sites of Rawalpindi and Islamabad. This high density was very logical and in association with heavy rain fall in the 1st and 2nd week of August in Rawalpindi and Islamabad which might have supported the rapid development of larval stages of this species leading to the higher densities in 3rd week of August. Sherin (2011) and Shakoor *et al*. (2012) have also observed unprecedented increase in dengue mosquitoes and dengue fever epidemic especially in the post monsoon period. On the other hand the very low density of this mosquito in Balakot mainly seems to be associated with the decrease in temperature, more steepness of the land and topography of the area, along with change in nature of vegetation, which does not support the breeding of this mosquito in this area.

A regular mosquito surveillance and vectorial capacity study is needed in these areas to keep a constant watch on this mosquito. Moreover, an awareness campaign for the visitors and tourists is recommended during pre and post monsoon season to avoid any untoward situation.

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Evaluation of Cadmium Profile of Blood Plasma in Lactating Cows: A Case Study

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Abstract.- The status of cadmium in blood plasma of cattle being reared at Khizerabad Livestock farm, district Sargodha, Pakistan was determined. The concentrations of Cd in plasma ranged from 0.52 to 0.70 mg/L, during various sampling intervals, respectively. The maximum levels of plasma cadmium in lactating cows indicated the potential threat of food chain contamination, which should be well thought-out as a potential source of toxicity for livestock and eventually for human.

Key words: Blood plasma, cows, semiarid environments.

Cadmium (Cd), a toxic and nonessential metal, is a widespread environmental pollutant that causes damage to kidneys and bone in humans (Jarup *et al.*, 1998; Tsuchiya, 1969). Cd is a potential toxin and pollutant that has no function in biological organism and is the most dangerous heavy metal for the environment due its low concentration and high mobility in organisms (Khattak and Khattak, 2013). Food is the major source of Cd exposure in the general population. In earlier experimental and epidemiological studies, the intestinal absorption of Cd was found to be increased whenever total body iron was depleted (Flanagan *et al.*, 1978; Kowal, 1988; Ragan, 1977). In non-contaminated, non-cultivated soils, cadmium concentrations are largely governed by the amount

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of cadmium in the parent material. Residual soils developed from shales have been reported (Lund *et al*., 1981) as having the highest concentrations with a mean of 7.5 mg/kg, whereas soils derived from sandstones and basalts have the lowest concentrations with a mean of 0.84 mg/kg. Cadmium is fairly immobile in the soil profile and tends to accumulate in surface soils due both to the adsorptive capacity of the humus content of surface horizons and from inputs from atmospheric deposition, fertilizers, and cycling through plants.

Cadmium is widely used in industry mainly in the manufacture of batteries, pigments, and stabilizers. It is frequently an impurity in zinc and therefore is found in a wide variety of consumer goods. It enters the environment, for example, from secondary zinc production, and from the use of zinc scraps, zinc pipes and gutters. Several other sources, *e.g.*, phosphate in fertilizer and fodder, wastes from phosphate ore processing, and sewage sludge contribute to the soil burden (Frieberg *et al.*, 1971)

The mechanism of absorption of Cd in the gastrointestinal tract is unknown. There is an increasing evidence that generation of high amount of reactive oxygen species takes place as result of Cd-toxicity in plants and animals (Xie *et al*., 2006; Amara *et al*., 2008), and bacteria (Achard-Joris *et al*., 2007).

Cadmium has no proven essential metabolic function although a few animal studies suggest that it may be needed in very small quantities (Schwartz and Spallholz, 1978; Perry *et al.*, 1977). The ion moves in the environment like calcium and zinc and accumulates from the soil in plants and from water in aquatic animals. It is cumulative in animals and recognized as very toxic (ATSDR, 1988). Indeed, on a mass basis, it is more toxic than lead.

Under normal soil conditions, plants take up only small quantities of cadmium from the soil but it is phytotoxic at much lower concentrations than metals such as Zn, Pb or Cu (Adriano, 1986; Kabata-Pendias and Pendias, 1984). Because it is available to plants from both air and soil sources, its concentration can increase rapidly in plants grown in polluted areas. Leafy vegetables and root vegetables are the most likely routes of cadmium supply to man because plants usually concentrate Cd in these areas (Kabata-Pendias and Pendias, 1984).

The environmental burden of cadmium due to anthropomorphic actions is increasing (Mielke *et al.*, 1991). There are therefore concerns about possible effects of long term low level exposures leading to the accumulation of cadmium in man (RCEP, 1983). No cases of Cd poisoning have been identified in Jamaica but there are several locations where Cd concentrations represent a potential hazard.

Rebuff figures are available about the Cd as an essential element. For example, the ruminants fed with a highly purified diet containing lower than 4 ppb Cd exhibited a growth depression (McDowell, 2003). Perry *et al*. (1977) fed animals a diet containing 13.7 ppb Cd and reported a higher weight with adequate Cd in Columbia. Reproduction was also adversely affected in female animals with no supply of Cd and those offspring born alive were less able to survive, compared to folks abounding an ample level of this element in the diet. Cadmium is toxic to virtually all systems in the body whether is ingested, injected or inhaled. Cadmium is inadequately absorbed but can mount up in muscle and can perturb the equilibrium of other minerals in the body (Phillips *et al*., 2004). The level of cadmium in the diet with 0.5 mg/kg is the higher tolerable amount which has been recommended for livestock (NRC, 2005). Because of the importance of cadmium, particularly toxic roles for livestock, the current investigation was planned to determine the levels of this element and to know its deficiency or excess in animal blood. Based on information as a result of this study, various supplements will be formulated to overcome the imbalance of cadmium in livestock to prevent the toxicosis in animals.

Materials and Methods

This study was carried out at an experimental centre at Khizerabad, Sargodha, with arid and semi-arid climate located in northwestern, Punjab at an altitude of 187 m. In this study, 20 cows of Sahiwal breed (5 cows at each sampling period) in their lactation phase with approximately the same weight (250-300 kg from a herd of 100 cows being raised on the centre. The age of the animals chosen for the study ranged from 40-45 months. These cows were

in lactation phase with 2nd lactation. The average temperature was 45±5°C during summer and 18±3°C in wintry weather. Relative moisture content was 85% in winter and 35% in summer. To assess the cadmium transfer from forages to cattle, blood samples from animals were collected from the livestock farm during October, 2008 to February, 2009. Daily consumption of forages by per individual ranged from 40 to 45 kg on fresh weight basis during this investigation. Five replicates were obtained after one month duration of animal blood during the study period.

Samples of blood were obtained from the jugular vein of the grazing cattle while the animals were in standing position without severely stressing them. An aliquot of blood (20 mL) was taken in a clean vial having heparin as an anticoagulant. The samples of blood were subjected to centrifugation to separate and harvest the plasma and stored at suitable temperature *i.e.* 20°C. Two ml of thawed plasma were wet digested with HNO_3 and $HClO_4$ in 1:1 ratio and made volume up to 25 mL by deionized water. For the chemical analysis of cadmium from the plasma samples atomic absorption spectrophoto-meter coupled with graphite furnace was used (AA-6300 and GFAEXi7i, Shimadzu, Japan).

The software of SPSS (20) was used for statistical analysis. One-way ANOVA was applied for the analysis of variance of data for plasma cadmium. Differences among cadmium levels at different sampling periods were worked out following Steel and Torrie (1980) at 0.05, 0.01 and 0.001 probability levels.

Results and discussion

Figure 1 shows the level of cadmium in the forage and blood plasma of lactating cows.

Blood plasma Cd concentrations in the present study varied consistently throughout the sampling periods with the trend to increase from first to fourth sampling periods, but the effect of sampling intervals on its concentration was statistically non-significant ($P \leq 0.05$). Plasma Cd concentrations varied from 0.52 to 0.70 mg/L and the lowest level was observed during 1st interval of sampling and the maximum level was found at the last sampling period (Fig. 1). These concentrations were considerably higher than those reported earlier by Gowda *et al*. (2000) in pastures and animals. One of the important features of Cd metabolism is the lack of an effective homeostatic control mechanism and retention of Cd in the body with an unnecessary long half life (McDowell, 2003). The amount of Cd in forages at this farm was high, and in blood plasma it showed little retention due to the fact that animals appeared to have no homeostatic control and fine mechanism to limit Cd retention to a nontoxic level (Miller, 1973), therefore, the amount of Cd in the body varies directly with the amount ingested (Miller, 1979). The findings of the present investigation suggested a low to severely marginal toxicity of Cd which was reflected through the analysis of blood plasma of animals. The specifically tailored mineral supplements are warranty recommended having higher concentration of those elements which are antagonistic to the Cd

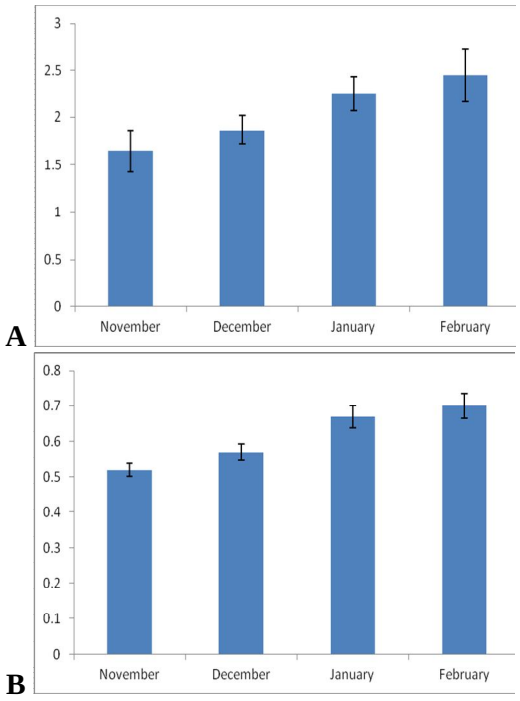

Fig. 1. Forage Cd concentration (mg/kg) (A) and blood plasma Cd concentration (mg/L) (B) at different time intervals. Lines on bars represent SE values. (Means are based on 5 samples per sampling period).

to the lactating cows and other livestock at the farm as a prophylactic measure to safe guard the animals from the potential Cd toxicosis.

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Growth Performance and Immune Status of Broilers Fed Graded Levels of *Albizia lebbeck* Seeds

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Abstract.- We conducted a study to use *Albizia lebbeck* seeds as alternative to canola meal on production performance and immune level in broiler chicks. A total of 150, day old broiler chicks were divided into four experimental groups *i.e.*, Al-0, Al-2, Al-4, Al-6 and Al-8 and were fed 0%, 2%, 4% and 8% *A. lebbeck* seeds in the diet of broilers, respectively. Each group was further divided into three replicates having 10 chicks per replicate in a completely randomized design. The birds were reared in separate pens for 40 days in an open sided house. Body weight and dressing percentage increased significantly in Al-2 and Al-4 groups. Antibody titer against the Newcastle and infectious bronchitis antigen was not affected between the control and treated groups. Based on the experimental findings, it was concluded that *A. lebbeck* could be used alternative to canola meal in broiler diet.

Key words: *A. lebbeck*, broilers, canola, production

Modern broiler chickens are fast growing species of poultry that are commonly raised to provide tender meat for human consumption. However, the rising cost of poultry feed has continued to be a serious problem (Khan *et al*., 2010). This is because the feed alone accounts for a heavy chunk of the total cost of production (Khan *et al*., 2010; Olorunsanya *et al*., 2010). To minimize the feeding cost, different feed additive or alternatives are used in poultry ration in order to achieve desirable effects (Khan *et al*., 2010; Khan *et al*., 2012a,b). *Albizia lebbeck* is grown naturally in Pakistan probably due to favourable environmental conditions and peculiar characteristics of the soil.

The species of *A. lebbeck* are found in the warmer parts of the world (Olorunsanya *et al*., 2010). The nutritive value of leaves, flowers of *A. lebbeck* is well known (Gupta, 1981; Pradhan and Dayal, 1981; Lowry, 1987; Schlink *et al*., 1991; Dwatmadji *et al*., 1992). It has been estimated that 100 kg of leaves yield 11-12 kg of digestible protein and 37 kg of digestible carbohydrates (Lowry, 1987). *A. lebbeck* seed has high protein and carbohydrate contents to the tune of 27.3 and 19.4% respectively (Muhammad *et al*., 2010). The seed is respectively (Muhammad *et al*., 2010). The seed is rich in magnesium, iron and selenium and could be a good source of these minerals in animal diet (Muhammad *et al*., 2010). Hassan *et al*. (2007) also reported *A. lebbeck* seeds and pods could be an important protein and minerals source for animals. Olorunsanya *et al*. (2010) concluded that supplementation of backed *A. lebbeck* seeds at the rate of 5% improved growth performance and carcass characteristics in broilers.

There are limited studies in broiler demonstrating the effect of *A. lebbeck* as a potential feed source. Therefore, this study was planned to use *A. lebbeck* seeds as alternative to canola meal on broiler performance and immunity.

Materials and methods

A total 150, one day old broilers were procured from the local market. The birds were kept under standard management conditions. Feed and water were given to the birds *ad libitum*. The birds were vaccinated against the prevailing diseases according to the recommended schedule for vaccination.

After a week of adaptation period the birds were divided into five groups i.e. Al-0, Al-2, Al-4, Al-6 and Al-8. *A. lebbeck* seed was added to replace canola seed meal in the ration of group Al-0, Al-2, Al-4, Al-6 and Al-8 at the ratio of 0, 2, 4, 6 and 8% respectively for forty days (Table I). The proximate analysis of the *A. lebbeck* shows moisture, 3.10±0.001; crude protein, 27.30±0.001; ether

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extract, 7.50±0.10; crude fiber, 38.50±0.01, and carbohydrate, 19.40±0.00. Each group had three replicates with 10 birds per replicate. Feed intake was calculated by subtracting feed refused from feed offered. Feed intake was calculated on daily basis. Weekly weight gain was calculated by subtracting initial weight from final weight of each week. Weekly feed conversion ratio (FCR) was calculated by divided weekly feed consumed by weekly weight gain. The experiment lasted for 40 days.

Table I.- Ingredients composition (%) of experimental diets.

Ingredients (%)	Al-0	Al-2	Al-4	Al-6	Al-8
Corn	39	39	39	39	39
Rice	12	12	12	12	12
Soyabean (46%)	16.5	16.5	16.5	16.5	16.5
Fish meal	4	4	4	4	4
Rice Polish	6	6	5.5	5	4.8
Canola meal	8	6	4	2	0
Albizia lebbeck	0	2	4	6	8
Gluten meal (27%)	2	2	2.5	3	3.2
Guar meal	4	4	4	4	4
Molasses	5	5	5	5	5
Dicalcium phosphate	2	2	2	2	2
Morble Chips	0.5	0.5	0.5	0.5	0.5
Salt	0.2	0.2	0.2	0.2	0.2
Vitamin + Mineral + Premix	0.25	0.25	0.25	0.25	0.25
DL Methionine	0.15	0.15	0.15	0.15	0.15
Lysine	0.3	0.3	0.3	0.3	0.3
Zincbacetracine	0.05	0.05	0.05	0.05	0.05
Coccidiostate	0.05	0.05	0.05	0.05	0.05
	100	100	100	100	100
Crude protein (%)	21	21	21	21	21
Metabolizable energy (Kcal per kg)	2900	2900	2900	2900	2900

Al-0 - control; Al-2 - 2% *A. lebbeck* seeds; Al-4 - 4% *A. lebbeck* seeds; Al-8 - 8% *A. lebbeck* seeds

*Provided per kg of diet: Mn 80 mg; Zn 60 mg; Fe 60 mg; Cu 5 mg; Co 0.2 mg; I 1 mg; Se 0.15 mg; choline chloride 200 mg; vitamin A (retinol) 12000 IU; vitamin D3 (cholecalciferol) 2400 IU; vitamin E (DL-α-tocopherol) 50 IU; vitamin K (menadione) 4 mg; vitamin B1 (thiamine) 3 mg; vitamin B2 (riboflavin) 6 mg; vitamin B5 (pantothenic acid) 25 mg; vitamin B6 (pyridoxine) 5 mg; vitamin B12 (cyanocobalamin) 0.03 mg; folic acid 1 mg

Blood samples on day 7, 15, 30 and 40 were collected for determination of antibodies titre against New castle disease (ND) and Infectious Bronchitis (IB). Antibody titre against ND and IB was determined using heamagglutination inhibition (HI) test as described by Alexander and Chettle (1977).

The data was statistically analyzed using standard procedure of analysis of variance, using one way ANOVA as described by Steel and Torrie (1981). The statistical package SAS (1998) was used for the data analyses. P value less than 0.05 was considered to be statistically significant.

Results

Feed intake, body weight, dressing percentage and FCR are given in Table II. Feed intake was not significant between the control and experimental groups. In group AI-8, the feed intake was higher, however, it was not transformed into the equivalent body weight. Body weight and dressing percentage improved significantly ($P<0.05$) in Al-2, Al-4 groups. The results also indicated that increasing the level of *A. lebbeck* in the feed by 6 and 8% had negative effect on the body weight and dressing percentage of the birds. Feed conversion ratio of the control and treated groups did not change significantly ($P>0.05$). The best FCR was observed in AI-4. Mean antibody titer against ND and IB is given in Table III. The results revealed the antibody titer against ND and IB were not affected by *A. lebbeck* seeds.

Table II.- Mean total feed intake (g), body weight (g), feed conversion ratio (FCR) and dressing percentage in control and treated broiler chicks fed different levels of *Albizia lebbeck* seeds.

Levels of *Albizia lebbeck* seed	Feed intake	Body weight	Feed conversion ratio	Dressing percentage
Al-0	3592.54	2247.70^{bc}	1.72	71.33^{ab}
Al-2	3681.27	2468.23^{a}	1.59	72.33^{a}
Al-4	3545.08	2473.50^{a}	1.51	73.66^{a}
Al-6	3497.25	2340.93^{ab}	1.53	67.66^{bc}
Al-8	3641.48	2123.06^{c}	1.77	65.33^{c}
SE	54.12	78.93	0.04	1.21
P value	0.076	0.043	0.091	0.041

*Al-0 - control; Al - 2: 2% *A. lebbeck* seeds; Al - 4: 4% *A. lebbeck* seeds; Al - 8: 8% *A. lebbeck* seeds

Discussion

Animal feeding is becoming costly due to the limitations posed by protein source. Therefore, the

search for protein-rich seed is an important process. One of the reasons is that the conventional protein sources are over-competed for animals (Laudadio and Tufarelli, 2011). According to the findings of Muhammad *et al*. (2010) *A. lebbeck* seed could be used as a potential source of protein in animal feed since it contains 27.3% crude protein.

Table III.- Mean antibody titer against Newcastle disease virus and infectious bronchitis virus in broiler fed different levels of *Albizia lebbeck* seed.

Levels of *albizia lebbeck* seed	Day 7	Day 15	Day 30	Day 40
New-castle disease virus				
Al-0	8.00	7.33	8.00	6.00
Al-2	9.00	7.00	7.66	6.33
Al-4	8.33	7.66	8.33	6.66
Al-6	7.33	8.33	9.33	7.00
Al-8	8.00	9.00	9.66	7.33
SE	0.03	0.04	0.01	0.01
P value	0.067	0.098	0.078	0.061
Infectious bronchitis virus				
Al-0	7.33	7.33	8.33	6.33
Al-2	8.00	8.00	7.33	7.00
Al-4	7.33	7.33	8.00	7.33
Al-6	8.00	8.00	7.66	8.00
Al-8	7.00	8.33	8.66	7.66
SE	0.33	0.33	0.33	0.33
P value	0.06	0.064	0.089	0.091

*For abbreviations see Table II.

Discussion

Animal feeding is becoming costly due to the limitations posed by protein source. Therefore, the search for protein-rich seed is an important process. One of the reasons is that the conventional protein sources are over-competed for animals (Laudadio and Tufarelli, 2011). According to the findings of Muhammad *et al*. (2010) *A. lebbeck* seed could be used as a potential source of protein in animal feed since it contains 27.3% crude protein.

In the present study, we observed that diet constituted with 2 and 4% *A. lebbeck* seeds improved the performance of the birds almost equally. Economically 2% *A. lebbeck* seeds incorporation is therefore recommended. Our results are similar to the findings of Olorunsanya *et al*. (2010) who found that 5% inclusion level of *A. lebbeck* improved the performance of the broiler. We also observed that increasing the level of the *A. lebbeck* seeds did not improve the performance of the birds. This might be due to the presence of tannin and saponins in *A. lebbeck* seeds which have negative affect on the feed intake and digestibility (Bate-Smith, 1973; Liener, 1989). Olorunsanya *et al*. (2010) concluded that increasing the level of *A. lebbeck* seeds above 5% may be due to the antinutritional elements which limit the useful effect of this plant. According to Esou *et al*. (1997) some legumes have thermo-labile and thermo-stable anti-nutrients which require extensive application.

According to Muhammad *et al*. (2010) the anti-nutritive components of this plant have various deleterious effects including reduced feed intake and bioavailability of minerals as well as indigestion problems which may lead to death. The toxic compounds are mainly concentrated in the seed especially the seed coat (Gill, 1992; Osagie, 1998). Muhammad *et al*. (2010) concluded that *A. lebbeck* seeds must be subjected to processing technique to use it as a source of protein and minerals. In the present study, therefore, we can conclude that higher levels had no positive effect probably due to the antinutritional components of *A. lebbeck.*

Literature review is limited on the immune potentiating effect of *A. lebbeck* in broilers and other animal models. From the present study, it is evident that unlike the deleterious effect of increasing levels of *A. lebbeck*, the immune response was not negatively affected. However, further experiments on the immune stimulating effects *A. lebbeck* are required.

In conclusion, a level of 2 and 4% *A. lebbeck* addition produced the best result in term of growth performance. Moreover, from an economical point of view, 2% *A. lebbeck* is recommended as alternative to canola meal in poultry diet.

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Interactive Effect of Nitrogen and Insecticide on Jassid, *Amrasca devastans* (Dist.) Population and Photosynthetic Capacity of Okra *Abelmoschus esculentus* (L.) Moench.

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Abstract- Infestation of phloem feeding insects reduce crop yield with or without reducing photosynthesis, however, in the present research, we report for the first time that jassid, *Amrasca devastans* (Dist.) infestation rate reduces crop yield by reducing photosynthesis. We assessed the interactive effect of nitrogen and *A. devastans* abundance on photosynthesis activity of okra *Abelmoschus esculentus (L.)* Moench. Insecticide spray reduced the jassid abundance on okra thereby improved growth of okra. Although increasing supply of N also improved the growth of insecticide sprayed or non-sprayed okra plants by enhancing photosynthetic pigments and photosynthetic efficiency, numbers of jassids increased significantly. However, this improving effect of N on growth was more in insecticide sprayed plants. Jassid infestation greatly reduced chlorophyll contents and photosynthetic rate, which is positively associated with jassid infestation-induced reduction in growth. Moreover, reduction in photosynthetic rate in okra plants due to jassid infestation may have been due to stomatal and some metabolic perturbations.

Key words: *Amrasca devastans*, jassid, Okra, *Abelmoschus esculentus.*

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Amrasca devastans (Dist.) is the regular insect pest of okra, *Abelmoschus esculentus* L., nymphs and adults of *A. devastans* suck from the surface and can cause 54-66% losses in yield (Dhandapani *et al*., 2003). *A. devastans* is also key pest of cotton and brinjal in the Punjab, Pakistan (Razaq *et al*., 2013; Yousafi *et al*., 2013). Application of nitrogen to plants changes the plant biochemistry that enhances infestation of homopteran insects (Jansson *et al*., 1991). Increasing N supply will result in improved photosynthetic activity of plants (Zafar *et al*., 2010). However, high N application enhances pest infestation. Therefore, present study was aimed to assess the interactive effect of nitrogen fertilization and insecticide spray on *A. devastans* abundance and photosynthetic activity of okra plants.

Materials and methods

Seeds of okra var Subz Pari were sown on beds (3.3 x 0.6 meter) with seed rate of 20 kg/ha in experimental fields' area of Bahauddin Zakariya University Multan during March 2011. Row to row and plant to plant distance was 60 cm and 30 cm, respectively. Nitrogen was applied in three different doses (0, 65, 130 and 260 kg/ha). Treatments were arranged in randomized complete block design with three replicates. Alternate rows in each plot were sprayed in each treatment to keep free from jassid infestation by spraying Jassper (Nytenpiram 20SL, Warble Private Limited) with a hand operated knapsack sprayer on 9th and 19th May. Jassid (adults and nymphs) numbers per plant were recorded weekly from appearance till crop maturation from four plants (upper middle and lower) of un-sprayed rows of each plot. Five plants from each treatment were analyzed for gas exchange characteristics using infra red gas analyzer (LCA-4, ADC, Hoddesdon, UK) whereas chlorophyll contents were measured with the help of SPAD-502 (Minolta, Japan).

Mean population of jassid and various photosynthesis parameters affected by N levels in were analyzed by analysis of variance (ANOVA). LSD and t-test were employed to sort out differences among treatments (Steel and Torrie, 1980).

Results and discussion

Numbers of *A. devastans* were significantly increased with increasing nitrogen level on all the sampling dates, however, jassid abundance were lower in June at all N levels (Table I). Application of 130 kg N ha^{-1} seems to be more promising as at this N dose jassid population was sufficiently lower to affect plant growth. Photosynthetic pigments and photosynthetic capacity of non-sprayed plants were lower than those spayed with insecticide. Moreover, increasing supply of N enhanced photosynthetic pigments and net CO_2 assimilation rate, particularly application of 130 kg N ha^{-1} was most effective. Regulation of photosynthetic capacity of okra plants is mainly associated with stomatal limitations as is evident from consistent increase in internal CO_2 with increase in photosynthetic rate

Table I.- Mean numbers of *Amrasca biguttula biguttula* (nymphs and adults) per leaf of *Abelmoschus esculentus* at Multan during 2011.

N kg/ha	Sampling dates						
	16-May	23-May	30-May	6-Jun	13-Jun	20-Jun	27-Jun
N0	05.16±0.8^{c}	05.78±0.6^{c}	1.59±0.4^{d}	1.20±0.2^{c}	2.61±0.5^{c}	05.92±0.8^{d}	11.61±1.1^{c}
N65	04.91±0.7^{c}	07.78±0.8^{c}	4.92±0.7^{c}	3.17±0.4^{b}	5.75±0.7^{b}	11.92±1.4^{c}	13.45±1.5^{c}
N130	09.11±1.4^{b}	13.89±0.8^{b}	7.28±1.1^{b}	4.44±0.7^{ab}	5.83±0.9^{b}	18.36±2.0^{b}	20.11±2.0^{b}
N260	23.77±2.3^{a}	18.61±2.2^{a}	8.92±1.2^{a}	5.53±0.9^{a}	9.16±1.2^{a}	27.55±2.7^{a}	31.38±3.0^{a}
F(df=3,6)	072.43*	026.19*	094.83*	020.35*	077.68*	138.98*	159.14*
P Value	0.0000	0.0008	0.0000	0.0015	0.0000	0.0000	0.0000
LSD values	3.61	3.96	1.13	1.43	1.05	2.72	2.50

Means followed by the same letters within a column are not statistically different at 5 % level of significance,* indicates significant

Table II.- Chlorophyll contents (%), photosynthetic rate, water use efficiency, transpiration rate and total internal CO_2 concentration of *A. esculentus* plants as affected by nitrogen levels and *A. biguttula biguttula* damage in sprayed and un-sprayed plots at Multan during 2011.

	Treatment	N0	N65	N130	N260	F-value	P-value	LSD-value
Chlorophyll content (%)	Sprayed	50.05±1.2d	55.39±0.7c	61.77±0.9b	70.69±1.3a	101.94*	0.00	3.04*
	Unsprayed	45.11±1.1b	48.75±1.0b	51.24±1.0ab	55.97±1.6a	006.36*	0.027	6.25*
	t-value	03.82*	06.19*	13.07*	04.57*			
Photosythetic rate (µmol CO_2 m^{-2} s^{-1})	Sprayed	12.68±0.6c	15.93±1.2b	21.66±1.0a	19.49±1.4a	34.49*	0.0004	02.33*
	Unsprayed	07.80±1.0	10.82±1.1	16.32±1.0	16.27±0.8	00.47^{ns}	0.00	30.84^{ns}
	t-value	3.9*	2.7*	4.6*	4.0*			
Water use efficiency (µmol CO_2 /mmol H_2O)	Sprayed	2.31±0.1c	3.55±0.1b	4.52±0.1a	4.39±0.1a	72.42*	0.00	0.414*
	Unsprayed	2.67±0.1c	3.86±0.1b	5.06±0.2a	4.78±0.1a	27.55*	0.000	0.709*
	t-value	2.7*	0.8^{ns}	1.4^{ns}	1.1^{ns}			
Transpiration rate (mmol H_2O m^{-2} s^{-1})	Sprayed	5.74±0.4a	4.46±0.3b	4.86±0.3b	4.48±0.3b	6.40*	0.026	0.82*
	Unsprayed	3.37±0.3	2.82±0.3	3.28±0.2	3.42±0.2	0.57^{ns}	0.00	1.27^{ns}
	t-value	3.8*	5.1*	5.5*	4.8*			
Total internal CO_2 concentration (µmol m^{-2} s^{-1})	Sprayed	337.37±1.2d	348.98±2.4c	360.28±2.0b	370.05±2.9a	85.39*	0.00	05.29*
	Unsprayed	329.99±4.6c	341.61±3.5bc	352.27±2.4ab	363.21±4.3a	05.25*	0.040	21.51*
	t-value	0.9^{ns}	2.2^{ns}	2.0^{ns}	0.9^{ns}			

Means followed by the same letters within a row are not statistically different at 5% level of significance (df=3). *and NS indicates significant and not significant F-value, respectively at 5% level of significance. A non significant value of t-test indicates that means in rows for each N level are not significantly different.

(Table II). These results can be related to earlier findings of Athar *et al.* (2011) who found that N nutrition enhance the photosynthesis by increasing N metabolism whereas enhanced infestation of whitefly to okra plants reduces photosynthesis which was partially attributed to stomatal limitations and partially with metabolic limitations. Similarly, feeding of *Bemisia tabaci* reduces chlorophyll contents and photosynthetic rate in tomato (Buntin *et al.*, 1993) and cotton (Lin *et al*., 1999). Though we did not report yield potential, however, it is suggested that small rise in net photosynthetic rate due to 130 kg N ha^{-1} application where jassid infestation is significantly low can translate into enormous increases in yield as has been observed earlier in wheat (Parry *et al.*, 2011). However, this aspect should be assessed in detail.

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Growth Promoting Activity of Crude Protein Extract of Ruminant Placental Peptides

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Abstract.- The placenta is a rich source of the peptide hormones, to cater the needs of the body during pregnancy, leading to a huge surge of physiological changes. It has been reported that the mammalian placenta produced placental growth hormone (during the last two trimesters replacing the normal growth hormone). Fresh placental tissue from *Bubalus bubalis*, *Bos indicus* and *Capra hircus* were collected and the total soluble proteins were extracted after washing and homogenizing in buffer pH 9.5. Total soluble proteins were isolated with ammonium sulfate after adjusting the at pH 8.0. The isolated peptide/protein extract from all the ruminant placentae were tested for growth promoting activity. It was found that administration of placental protein extract resulted in increase in the body weight and dimensions of the long bones, compared with the control subjects. It was also noticed that bubaline placental extract enhanced the growth at a higher rate compared with those of goat and cow.

Key words: Placental hormones**,** *Bubalus bubalis, Capra hircus, Bos indicus,*

Placenta is an organ which develops during gestation. It produces a large number of proteins during gestation. Growth hormone (GH), prolactin, somatolactin, and related mammalian placental hormones including placental lactogen (PL), form a family of polypeptide hormones which share structural similarities and biological activities (Forsyth and Wallis, 2002; Kohmoto and Bern, 1970; Buttle and Forsyth, 1976; Yamamoto *et al.*, 2011). Bovine placental lactogen (bPL) has been implicated as a luteotropic agent, and is also capable of stimulating mammogenesis and lactogenesis (Anthony *et al.*, 1995). bPL is a glycoprotein hormone that has lactogenic and somatogenic properties. Bovine placenta and bPL have characteristics that are different from those of ovine and caprine species, whereas the histological architecture of the placenta, molecular structure of PL and plasma PL profiles are closely related in sheep and goats (Takahashi, 2006). bPL secreted from the placenta seems to be heterogeneous. PL is a protein which is produced by pre-pro-bPL of 236 amino acid residues with a 36 amino acid signal peptide (Schnler *et al.*, 1988). bPL has consensus N-glycosylation site and many potential sites for O-glycosylation, where carbohydrate chains are added (Duckworth *et al.*, 1986; Soares *et al.*, 1998*)*. The bPL isoforms with different molecular weights and charges have been reported (Byatt *et al.*, 1986, 1990). Two dimensional gel electrophoresis shows the presence of bPL with two different molecular weights (29,000 and 32,000) (Byatt *et al.*, 1990). Colosi *et al.* (1982) reported the purification and characterization of mouse PL. Kessler and Schuler (1991) reported the presence of truncated bPL transcripts comprising 13% of total bPL mRNA. In addition to this, bPL isoforms with the same molecular size and different PI values were also found to be present in placental tissues (Byatt *et al.*, 1986, 1990). The bPL mRNA is transcribed in trophoblast binucleate cells *(*Yamada *et al.*, 2002) and synthesized bPL protein is stored in membrane-bound granules in these cells (Wooding, 1982). The mRNA encoding bPL is first detectable with *in situ* hybridization in trophoblast binucleate cells at about day 20 of gestation (Flint *et al.*, 1979; Yamada *et al.*, 2002).The expression of bPL lasts till the end of gestation; the bPL message is still detectable in cotyledonary tissue collected immediately after calving (Ushizawa and Hashizume, 2006). The plasma levels of bPL are comparable to the quantity of bPL transcripts in the placental tissue. Patel *et al.* (2004) reported that the transcription level of bPL in placentomes increased with the progression of gestation, and was still maintained at parturition. Changes in the plasma bPL concentration during

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gestation are relatively minor and more gradual than those of placental steroids (Takahashi *et al.*, 1997; Patel *et al.*, 1999). The half-life of recombinant bPL derived from *E. coli* was estimated to be approximately 7.5 min (Byatt *et al.*, 1992a), although the half-life of native bPL synthesized by the placenta could possibly be longer than that of recombinant bPL because it is glycosylated. So, the short half-life of bPL is one of the main factors behind the gradual increment in plasma level (Green *et al.*, 1998). PL shares the functional homology with GH and prolactin. Singh *et al.* (1992) reported that ovine PL is a potent somatogen in GH-deficient rats, after comparing the somatogenic activity of PLG and bGH. Ovine PL has proven to be potent anabolic and lipolytic agent in the dwarf rat. Byatt *et al.* (1992b) reported the binding of PL to somatogenic and lactogenic receptors. Byatt *et al.* (1997) reported the increase in milk yield. Leibovich *et al.* (2001) have shown effect of recombinant ovine placental lactogen and recombinant ovine GH on growth of lambs and milk production of ewes. Circulating concentrations of ovine PL were 24.6±1.6 ng/ml on day 5 for ewes treated with ovine PL, but concentrations of ovine PL were undetectable in ewes treated with either saline or bGH (Min *et al.*, 1997).

It is evident that the placental peptides have biotechnological importance in the dairy industry. Administration of crude extract of placental proteins and peptide to the farm animals may provide a route to farmers, to direct benefit. Here we have compared the somatogenic activity of placental peptides of three farm animals.

Materials and methods

Tissue samples of placenta were collected from the local species of water buffalo, cow and goat from Lahore, Pakistan. The placental samples were washed with distilled water and homogenized in a buffer pH 9.5 to solubilize the proteins. The protein extract was centrifuged at 10,000xg for 30 min to remove the insoluble material. The clear protein extract was concentrated with 80% ammonium sulfate incubated overnight at 4°C. The precipitates were separated by centrifugation and then dissolved in buffer pH 8.0. Ammonium sulfate was removed after dialysis and protein solution was used as a source of placental peptides/protein extract for biological assays. Soluble proteins in the supernatant were estimated by Brafdord methods using casein as standard.

Three sets each of three male adult Swiss albino mice, *Mus musculus* (age-30 days) were injected with 3µg/g body weight of the placental protein for 8 weeks. Another set of three male adult mice as control animals was injected with 0.05M Tris HCl (pH 8), without protein for 8 weeks. All the mice were monitored for physical health and diet intake. The body weight was recorded every week. After 8 weeks the mice were killed, long bones were separated after dissection, cleaned and stored in formalin. The lengths and width of bone ends of the control as well as treated mice were measured with the help of calipers.

Results and discussion

Figures 1-3 show the effect of exogenously administered placental peptides/proteins for 8 weeks on the total body weight, and length and breadth of tibia bones of male adult mice, *Mus musculus*. The increase in body weight (Fig. 1) and increase in length and breadth of tibia bone (Fig. 2) in placental protein/peptide-treated mice compared to their respective controls is indicative of growth promoting activity of the placental extract.

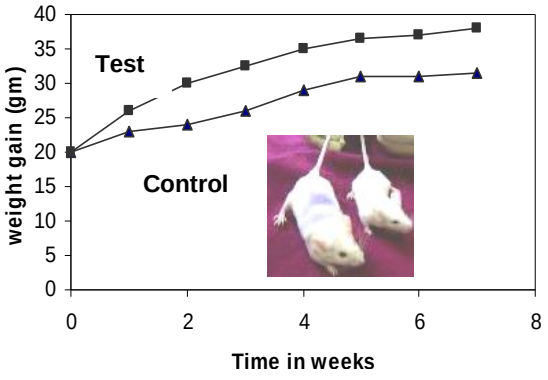

Fig. 1. The effect of exogenous placental placental peptides on mice for seven weeks. The mouse marked with blue colour was injected with placental peptides at the concentration of 3µg/gm body weight per day for 8 weeks. The control was injected with the buffer without placental peptides. The test animal is marked with blue, the control is white in colour. The experiment was carried out in triplicate.

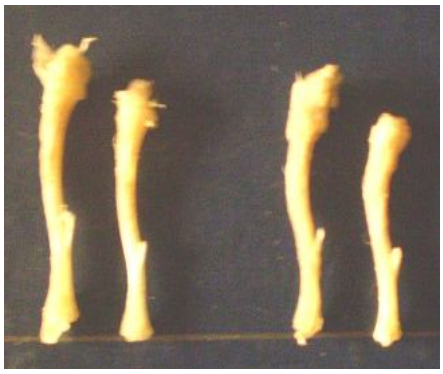

Fig. 2. Effect of placental peptides on dimensions of tibia of Swiss albino mice. Injection of growth hormone at 3 µg/ml for 8 weeks resulted in a increase in bone length (A,C). Tibia of untreated mice is shown in B and D.

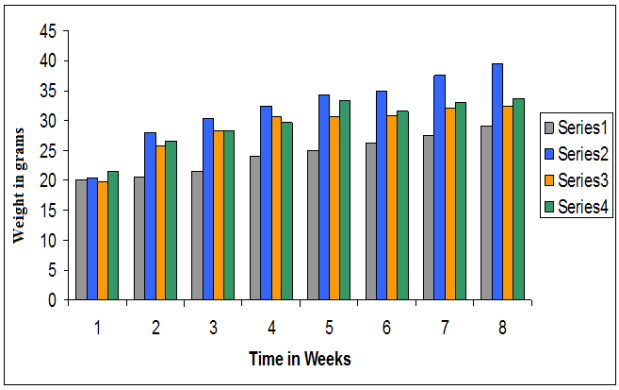

Fig. 3. Effect of placental peptides on the growth rate of mice. An increase in growth rate is shown compared to the normal subject. Series 1, control; Series 2, placental peptide extract from *Bubalus bubalis*; Series 3, placental peptide extract from *Bos indicus* and Series 4, placental peptide extract from *Capra hircus.*

Results reported are in agreement with those previously reported for placental peptides (Singh *et al.*, 1992).

The placental protein extract of all the three ruminants *viz.*, cow, goat and water buffalo were found to cause an increase in the growth rate in the mice. Byatt *et al*. (1992) have suggested binding of placental lactogen to somatogenic and lactogenic receptors to triggers growth promoting response. Growth promoting effects of recombinant ovine PL and recombinant ovine GH has been reported in lambs and milk production of ewes (Leibovich *et al.*, 2001). It was recorded that bubaline placental extract was more potent than the extract of goat and cow. It was concluded that the placental extract could increase the growth rate in mammals. Detailed investigations are required in this regard, before using the placental extracts as growth enhancer in farm industry.

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A New Record of *Paratylenchus lepidus* (Nematoda: Tylenchulidae) Associated with Ramie Root in Yuanjiang, Hunan Province, China

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Abstract.- In this paper, for the first time, we report a new record of *Paratylenchus lepidus*. The nematode was isolated from rhizosphere of ramie in Yuanjiang, Hunan province, China. The morphological characters of this population are very similar to *P. lepidus* previously reported from Taiwan. Additionally, ribosome DNA internal transcribed spacer (ITS) sequences of this population showed 85% identity with that of *P. lepidus* from Taiwan.

Key words: Ramie, pine nematode, morphology, ITS.

Ramie (*Boehmeria nivea* L. Gaud), also called "China grass", is a herbaceous perennial plant mainly planted in China, India and other Southeast Asian and Pacific Rim countries (Liu *et al*., 2013). For thousands of years, ramie is well known for its excellent fiber. More recently, its leaves and shoots were found to be good feed for goose and cow. As perennial plants, the storage roots of ramie are fat and fleshy and are attractive to larvae of cockchafer (*Anomala corpulenta* Motschulsky, *Anomala exoleta* Faldermann) - popularly known as white grub, larvae of *Paraglenea fortunei* (Saundeas) and nematode (Yu *et al*., 2011; Liu *et al*., 2012; Zhuo *et al*., 2013).

In order to investigate plant parasitic nematode associated roots of ramie, a survey was performed in four provinces (Hunan, Hubei, Sichuan and Jiangxi) and a municipality (Chongxing) of China, in 2010 to 2011. Pin

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nematodes were isolated from samples from Yuangjiang of Hunan, Dazhou of Sichuan and Fuling of Chongqing. Among them, abundant pin nematodes were isolated from Yuanjiang's samples. The nematodes were preliminary identified as *Paratylenchus* according to body traits, such as fat, short body and long stylet (Liu *et al*., 2012). Pin nematodes are known to live outside of the plant roots and only feed epidermic cells. Although the pin species doesn't result in serious yield loss of ramie, the wound it made would facilitate the subsequent infection of other soil-borne pathogens, such as *Rosellinia necatrix*.

Identification of the pin nematode was mainly based on characters of morphology and rDNA-ITS sequence. Chen *et al*. (2007, 2009) identified *P. lepidus* from tea plantations and *P. minutus* associated with sugar apple, sweet orange and buntan, based on morphology traits and rDNA ITS sequence. Riga *et al*. (2009) described yield reduction of dryland peas and lentils caused by nematode, and the causal agent was identified as *P. hamatus* by morphological traits. More recently, *P. labiosus* from different geographical areas in the continental United States were identified according to morphological and molecular characters (López *et al*., 2013).

In the present study, we described pin nematode *P. lepidus* from rizosphere of ramie. The nematode was identified with morphology traits combined with rDNA-ITS sequence. To our knowledge, this is the first report of *P. lepidus* parasitic on ramie.

Materials and methods

Sampling and nematode extraction

Soils in 20 to 30 depths near the roots of ramie planted in experimental fields in Yuangjiang, Hunan province were sampled. Nematodes from soil were extracted with the method as described by Anwar *et al.* (2012). Pin nematodes were picked under a stereomicroscope and washed twice with distilled water. The population was designated as YJ2012.

Morphological study

Nematodes for morphological study were heat killed, fixed in TAF and processed in slow glycerin process. Photomicrographs were taken with a digital camera linked to a computer, and measurements were made with the aid of imaging software (Olympus DP-soft).

DNA extraction and PCR application

DNA of single nematode was extracted by proteinase K technique as described by Yu *et al*. (2011) with minor modification. Single nematode was firstly surface sterilized with 70% ethanol for 5 min and washed twice with double distilled water. Then the single nematode was transferred into a 200 µl PCR tube (containing 8 µl double distilled water and 1 µl 10×PCR Buffer). The tube was submerged in liquid nitrogen for 3-5 min, subsequently incubated at 85°C for 3 min, then put in ice and 1 µl of proteinase K (2 mg/ml) was added. The mixture was incubated at 56°C for 15 min and 95°C for 10 min in a thermal cycler. The mix was then centrifuged for 2 min at 14,000 r/min, the supernatant were used as template for PCR amplification. For replicates, 10 nematodes were used to extract DNA, respectively.

For PCR amplification, a pair of universal primers, Pv1 (5'-ttgattacgtccctgcccttt-3') and Pv2 (5'-tttcactcgccgttactaagg-3') within the conserved region (Vrain *et al*., 1992) of rDNA internal transcribed space (ITS) sequence were used. PCR were performed in a total volume of 15 ul containing 7.5 µl 2×masterMix (Beyotime, Nantong, China), 0.6 µl each primer (10 mmol/l), 2.3 µl sterilized double distilled water and 4 µl DNA. All PCR reactions were performed in a C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA) with an initial denaturation at 95°C for 4 min, followed by 34 cycles of 95°C for 45 sec, 50°C for 45 sec, 72°C for 1 min and a final extension of 10 min at 72°C. PCR products were analyzed with electrophoresis on 1.5% agarose gel.

Sequencing and analysis

Mini gel containing amplification fragments were excised with a mobile UV light. The DNA fragments were purified from gel with a Universal DNA Purification Kit (Tiangen, Beijing, China) and then cloned into pMD-18 vector (Takala, Dalian, China) and later transformed into *Escherichia coli* DH5*α* competent cells (Tiangen, Beijing, China).

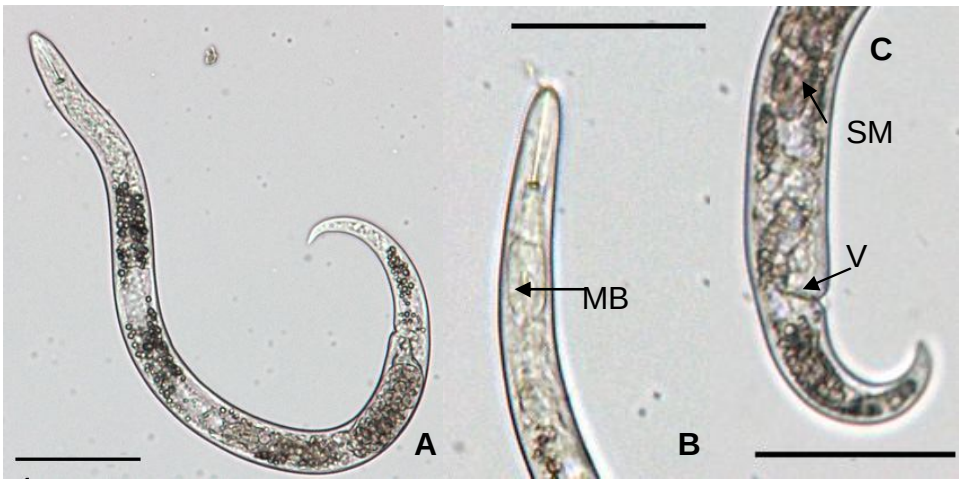

Fig. 1. Photomicrographs of female *P. lepidus* from Yuanjiang. A, whole body; B, Anterior region (MB, median bulb); C, Posterial region (SM, spermatheca; V, vulva). Bars: A-C, 50 µm.

Transformed cells were placed onto LB plates containing ampicillin, X-gal and IPTG and incubated at 37°C for overnight. After blue-white selection, positive bacterial colonies identified by colony PCR with the primers as described above to exclude false positive. The resulted positive bacterial colonies were incubated in LB liquid containing ampicillin at 37°C for overnight. Recombinant plasmids were extracted with Plasmid purification mini kits (Tiangen, Beijing, China) and sequenced in Shanghai Sunny Biotechnology Co., Ltd.

Results and discussion

Morphology

Female: After killed by gentle heat, female body curved as "C" shape. The body is characterized by obvious stylet, median bulb, vulva in posterior region and tapering tail (Fig. 1). Lip's anterior end is flat and there is no annule. No clear line between the posterior region and head was observed. Median bulb is oblong shape, with obvious valve gate. There are no clear division between esophageal gland and intestine. Anus open is very small and not easy to see. Tail's shape shows some variations, gradually thin end like oxhorn (Fig. 1A) or suddenly thin end like fishhook (Fig. 1C).

Morphological measurements are presented in Table I. The morphological characters of YJ2012 are similar to those of *P. lepidus* as described by Raski (1975) and of different population described by Chen *et al*. (2007), except for the a (body length / body width). Nematode YJ2012 is thinner than the paratypes and Taiwan population.

Male: not found.

Table I.- Comparison between the morphometrics of *P. lepidus* associated ramie and paratypes.

Characters [+]	Origin	
	Yuanjiang	Paratypes[§]
n	26	21
L	0.35±0.04 (0.26-0.42) [-]	0.33 (0.28-0.40)
a	18.46±2.22 (13.18-23.17)	25 (23-31)
b	4.17±0.52 (3.39-5.14)	4.0 (3.4-4.6)
c	12.87±2.1 (5.39-10.36)	25 (22-27)
V	79.05±3.67 (18.09-84.94)	82 (80-84)
T	29.49±7.6 (19.53-38.89)	19.53-38.89
Sty	23.98±3.8 (13.73-27.91)	25 (22-27)

[+] n, number; L, body length (mm); a, body length / maximum width; b, body length / the distance from head end to posterior end of esophageal gland; c, body length / length of tail; V, ratio between distance from vulva to anterior end of body and total body length in %; T, tail length (µm); Sty, Stylet (µm).
[-] Measurements in form: mean ± standard deviation (range).
[§] Raski, D. J. (1975)

Molecular identification

The genomic DNA of ten individual nematodes was used as template for PCR amplification with primer Pv1 and Pv2, respectively. The PCR products (about 1000 bp)

were subjected to agarose gel electrophoresis and visualized under UV light (Fig. 2). The amplified fragments were then excised, recovered and sequenced. The yielded two ITS sequences were deposited in GenBank with the accession nos. JX992859.1, and JX992860.1.

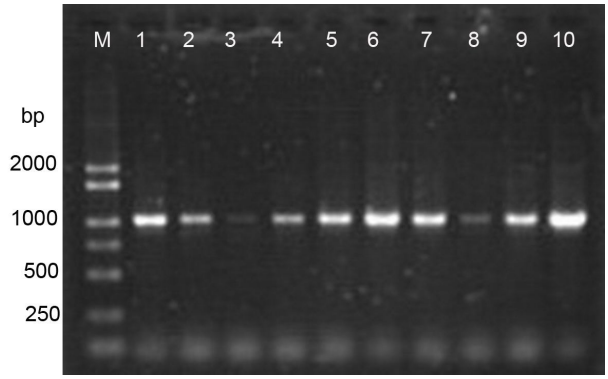

Fig. 2. PCR amplification products of rDNA ITS region of nematodes. M, DGL 2000 DNA ladder; Lanes 1 to 10, Amplification fragments of ITS sequences from ten nematodes.

The ITS sequences were searched against nr databases in GenBank using BLASTn analysis. The results showed that ITS sequences of YJ2012 associated ramie roots have 88% nucleotides identity with *P. minutus* (Chen *et al*., 2009) and 85% nucleotides identity with *P. lepidus* (Chen *et al*., 2007), respectively.

According to the morphological characters and ITS sequences similarity, *Paratylenchus* spp. from Yuanjiang associated ramie roots was identified as *P. lepidus*. To our knowledge, this is the first report of *P. lepidus* from rhizosphere of ramie.

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Assessment of Biological and Physical Environmental Risk Factors of Sarcoptic Mange in Pet Dogs*

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Abstract.- The present study was aimed at assessing the prevalence of sarcoptic mange in pet dogs and to investigate the cumulative role of biological factors such as age, gender and breed of the dog and physical environmental factors such as temperature, relative humidity and rain fall in the spread of this highly contagious parasitic disease. Sarcoptic mange was detected in 10.25% of the 3621 dogs brought to different veterinary clinics of Lahore for treatment of different ailments from January 2010 to December 2010. The dogs less than one year old were more heavily infested (12.21%) compared to those more than one year of age (7.36%). Both male and female dogs were found to be equally susceptible. Among the various breeds, German Shepherd showed the maximum prevalence (12.44%) and Doberman showed the minimum prevalence (6.10%). Maximum prevalence was noted in winter months and after the heavy rainfall of the previous months.

Key words: *Sarcoptes scabiei,* itch mite, scabies.

Sarcoptes scabiei, the itch mite is an ectoparasite which causes scabies in human worldwide. The same species of mite also infests a variety of wild and domestic mammals and causes sarcoptic mange in them (Pence and Ueckermann, 2002; Fitzgerald *et al*., 2004). More than 15 varieties of *S. scabiei* have been described from different hosts. These varieties, though morphologically indistinguishable, are physiologically and genetically distinct (Fain, 1978).

Sarcoptic mange in dogs or canine scabies it is caused by *Sarcoptes scabiei* var. *canis*. It is one of the most common ecto-parasites reported in dogs (Chang *et al.*, 1990). Although canine scabies is normally not a life threatening disease, but if left untreated, it may become chronic and cause severe discomfort to the infested animal. The mite can infest humans and produce severe pruritic skin lesions (Hewitt *et al*., 1971*;* Burroughs and Elston, 2003).

In Pakistan only a few reports are available regarding sarcoptic scabies in pet animals. A study conducted at Faisalabad showed a very high prevalence (37.5%) of sarcoptic mange in pet dogs (Irfan *et al.,* 2003). Some studies on sarcoptic mange in cattle and sheep are available which show a high prevalence of sarcoptic mange in farm animals in Pakistan (Qudoos *et al.,* 1997; Aatish *et al.,* 2007).

The study was aimed at investigating the role of biological factors such as age, sex and breed of the dogs in the spread of the disease, and recording the month wise and season wise prevalence of sarcoptic mange in pet dogs and to co-relating it with the physical factors of environment such as temperature, humidity and rainfall.

Materials and methods

The study was conducted from January 2010 to December 2010 in Lahore (31.15° – 31.45° N, 74.01°–74.39°E). A total of 3621 dogs brought to different veterinary clinics of Lahore for different ailments were thoroughly examined for the presence of symptoms of mange. These symptoms are haemorrhagic lesions with scales and serous exudates on different parts of body. The information regarding age, sex and breed of each dog was maintained.

The skin scrapings were collected from 426 suspected dogs, edges of the lesions in clean sterilized Petri dishes. These samples were brought to the laboratory for examination. Petri dishes containing scrapings were warmed at 38°C for approximately 2 min and then examined under the stereoscopic microscope for the presence of the various stages of the life cycle of mites.

The negative scrapings were transferred to the test tubes containing 10ml of 10% KOH solution

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and were heated for 5 minutes in a beaker containing boiling water. The tubes were centrifuged for 5 min at 2000 rpm and the supernatant was discarded. About 5 ml of water was added to the sediment and the tubes were centrifuged again. The supernatant was again discarded and the sediment was examined microscopically for the presence of mites and their developmental stages (Soulsby, 1982).

For recording season-wise prevalence, the year was apportioned with the following break-up: Winter (November-February), Spring (March-April), Summer (May-August) and Autumn (September -October)

Prevalence of sarcoptic mange in relation to physical environmental factors, such as temperature, relative humidity and rainfall was also calculated. The meteorological data was obtained from the Meteorological Department, Lahore station. Month-wise prevalence of canine scabies was correlated to mean monthly temperature, mean monthly relative humidity and total monthly rainfall to investigate the role of these physical environmental factors in the spread of this highly contagious disease. Correlations and data analysis was carried out using Graph Pad Prism version 4.00 for Windows (2003), Graph Pad Soft Ware, San Diego California USA, (www.graphpad.com). $P<0.05$ was considered statistically significant.

Results

From a total of 3621 pet dogs, the skin scrapings were examined microscopically in 426 suspected cases, to confirm the presence of *S. scabiei*. Of these 371 dogs were found positive for sarcoptic mange. The overall mean prevalence of sarcoptic mange obtained in pet dogs was thus 10.25%.

The overall infection rate was analyzed by age, gender and breed of the pet dogs.

Age: The age of pet dog positive for mange included in the study ranged from 4 days old to 11 years old. The dogs less than one year of age were more heavily infested (12.21%) compared to the dogs more than one years of age (7.31%) (Table I).

Gender: Both female (98) and male (273) dogs were found equally susceptible. The prevalence of sarcoptic mange was slightly higher (10.44%) in the female compared to the male (10.18%) dogs (Table I).

Table I.- Age and sex- wise prevalence of *sarcoptic mange* in pet dogs.

Age/sex/breed	No. of dogs examined	No. of positive dogs	Prevalence (%)
Age (Years)			
< 1 year	2154	263	12.21
>1 year	1467	108	07.36
Total	**3621**	**371**	**10.25**
Sex			
Females	939	98	10.44
Males	2682	273	10.18
Total	**3621**	**371**	**10.25**
Breed			
German shepherd	1897	236	12.44
Russian	526	47	8.94
Rot weiller	257	20	7.78
Labrador	368	**26**	**7.07**
Doberman	295	18	6.10
Cross breed	278	24	8.63
Total	**3621**	**371**	**10.25**

Breed: The maximum prevalence was detected in German Shepherd (12.44%) and the minimum prevalence was detected in Doberman (6.10%). When only positive cases were considered, German Shepherd showed 63.61% of the total infected animals **(**Table I).

Physical factors of environment as risk factors: The month-wise prevalence of sarcoptic mange shows that maximum prevalence (15.76%) was recorded in the month of January while the minimum infestation rate (6.94%) was recorded in the month of July (Table II).

The prevalence showed a marked seasonal pattern. The highest prevalence of sarcoptic mange was recorded in winter months (13.37%), followed by spring (11.42%) and autumn (9.87%). The minimum prevalence was noted in summer season (7.57%) (Table II).

The meteorological data obtained from Meteorological Department of Pakistan Lahore station was used to investigate the role of physical factors in the spread of the disease. Statistical

Table II.- Month-wise and season-wise prevalence of *Sarcoptic mange* in pet dogs during the year 2010.

Months	No. of dogs examined	No. of positive dogs	Prevalence (%)
Month-wise			
January	311	49	15.76
February	231	32	13.85
March	256	31	12.11
April	252	27	10.71
May	357	26	07.28
June	431	31	07.19
July	346	24	06.94
August	279	26	09.32
September	335	38	11.34
October	273	22	08.06
November	256	27	10.55
December	294	38	12.93
Total	**3621**	**371**	**10.25**
Season-wise			
Winter (Jan-Feb, 2010) (Nov-Dec, 2010)	1092	146	13.37
Spring (March-April, 2010)	508	58	11.42
Summer (May-Aug, 2010)	1413	107	07.57
Autumn (Sep-Oct, 2010)	608	60	09.87
Total	**3621**	**371**	**10.25**

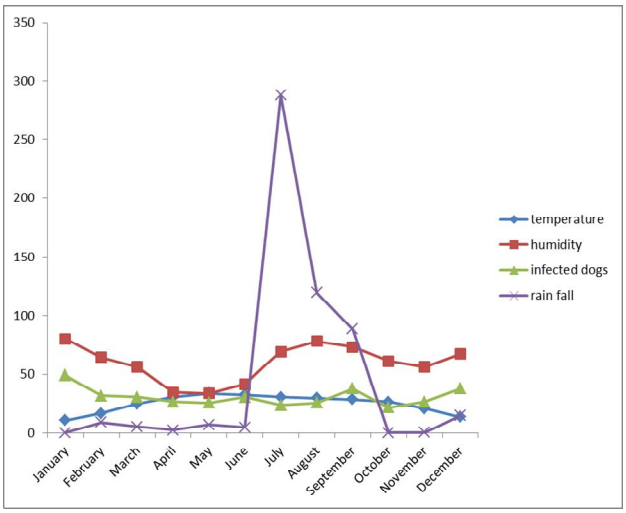

Fig 1. Showing the relationship between ambient temperature (C), relative humidity (%), rainfall (mm) and infection (%).

analysis of data reveals a significant negative correlation (r=-0.8668, P=0.0003) between monthly prevalence of infestation and mean monthly temperature. No significant correlation was found between monthly prevalence of disease and mean monthly relative humidity(r=0.4526, P=0.1396).The correlation between monthly prevalence of disease and the total monthly rainfall was non- significant (r=-0.3864, P=0.2147), however, a marked increase in the prevalence was noted after heavy rainfall of previous months. A prominent increase in the prevalence of sarcoptic mange in the months of August and September was noted after the heavy rainfall in the months of July (288mm) and August (119.4mm) (Fig. 1).

Discussion

In this study, the age of the pet dog was found to be a risk factor. The data clearly indicate that the young dogs less than one year of age were predominantly affected. Among the cases, 70% (263/371) dogs were under the age of one year. The study carried out by Curtis and Paradis (2003) indicated no influence of age on the prevalence of the disease. However, the study conducted by Feather *et al.* (2010) showed 40% prevalence in dogs less than two years of age among the cases and suggested that more sociable behavior of puppies with other dogs and their greater tendency to explore outdoor environment was the main cause of such a high prevalence of scabies in young dogs. Arlian *et al.* (1996) investigated that 88% of the older dogs develop immunity after the first exposure to the mite and chances of re-infestation are decreased. In another study Arlian and Morgan (2000) found that although 58% of the young dogs contained immunoglobulin G (IgG) and immunoglobulin E (IgE) against the proteins of *S. scabiei* var. *canis* but this may be due to cross-reactivity of the antigenic epitopes of *S. scabiei* with house dust mite antigens. They suggested that very young dogs actually have a very little or no immunity at all, so they have greater chances of

contracting canine scabies. The similar trend of disease is also reported by many researchers in other companion animals. In a study conducted on buffaloes by Hayat *et al.* (1996) in Faisalabad showed a significantly high prevalence (94.02%) of sarcoptic mange among the calves of less than one year of age as compared to the animals more than one year of age (5.98%).

The data collected in this study showed the equal susceptibility of both sexes. The previous studies confirm this findings and sex is not reported as a risk factor in dogs to contract infestation (Curtis and Paradis, 2003). Moreover, high prevalence of Sarcoptic mange in German Shepherd among the cases is due to the fact that the German Shepherd is the most favorite breed kept as pets in Pakistan and unluckily it is the most susceptible breed also.

The climatic factors definitely seem to be involved in the dynamics of the disease. The disease is more readily transmitted in wet and cold weather, so it is more prevalent in winter as compared to summer. Qudoos *et al.* (1997) while working on sarcoptic mange in sheep in highland Balochistan (Pakistan), investigated that the temperature range between19-24 was the optimum range for the propagation of parasite.

In the dislodged mite, the viability is directly affected by the temperature and relative humidity. High relative humidity and low temperature generally favor longer survival of the dislodged mites by reducing desiccation (Fain, 1978; Arlian *et al.*, 1984, 1988; Ibrahim and Abu-Samra, 1987). While working on Ibex population in Sierra Nevada, Perez *et al.* (1997) investigated that prevalence of scabies was extremely low in dry years even in the areas where scabies was endemic in wild Ibex population. Rainy season exacerbates the transmission of the disease. Vyrypaev (1985) also emphasized the role of rainfall in the speedy propagation and longer survival of the dislodged mites.

It is concluded that the climatic conditions in Pakistan and the poor management of the companion animals are mainly responsible for such a high prevalence of sarcoptic mange in pet dogs.

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