Short Communications

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COMPARATIVE ERYTHROCYTE SIZE AND MORPHOLOGY OF SOME LACERTID LIZARDS FROM TURKEY

Abstract.- General morphology and size of erythrocytes of the some lacertid lizards, *Lacerta trilineata, L. viridis, L. taurica* and *L. sicula* (Lacertidae), living in Turkey was studied using Wright's technique. The longest erythrocytes were found in *L. sicula* and the shortest ones in *L. trilineata.*

Key words*:* Erythrocyte morphology, Size, *Lacerta*, Lacertidae.

The first studies on the blood of reptiles described the structures, often comparing them with those of the other vertebrates (Saint Girons, *Biology of the Reptilia*, Vol. **3**, pp. 73-91, 1970).

Various authors have described the circulating blood cells of different reptile species (Ryerson, *J. Ent. Zool*., **41:** 49-55, 1949; Hartman and Lessler, *Biol. Bull*., **126:** 83-88, 1964; Szarski and Czopek, *Bull. Acad. Pol. Sci. Cl. II. Ser. Sci. Biol*., **14(6):** 437-443, 1966; Saint Girons, *Biology of the Reptilia*, Vol. **3:** 73-91, 1970; Canfield and Shea, *Anat. Histol. Embryol*., **17:** 328-342, 1988; Cannon *et al., Anat. Histol. Embryol*., **25:** 11-14, 1996; Alleman *et al*., *Am. J. Vet. Res*., **60:** 507-514, 1999; Sevinç *et al*., *Turk. J. Zool*., **24:** 207-209, 2000; Sevinç *et al*., *Asiatic Herpetol. Res*., **10:** 217-223, 2004; Sevinç and Uğurtaş, *Asiatic Herpetol. Res*., **9:** 122-129, 2001; Arıkan *et al*., *Amphibia-Reptilia*, **25(4):** 465-470, 2004 ; Tosunoğlu *et al*., *Asiatic Herpetol. Res*., **10:** 230-234, 2004; Martinez-Silvestre *et al., Res. Vet. Sci.*, **78:** 127-134, 2005; Carvalho *et al*., *Comp. Clin. Pathol*., **15:** 169-174, 2006, Metin *et al*., *Acta Vet. Brno*., **75:** 49-55, 2006).

There are few studies on reptilian blood cells in Turkey (Sevinç *et al*., *Turk. J. Zool*., **24:** 207-209, 2000; Atatür *et al*., *Turk. J. Zool*., **25:** 149-152, 2001; Sevinç and Uğurtaş, *Asiatic Herpetol. Res*., **9:** 122-129, 2001; Uğurtaş *et al*., *Zool. Stud.*, **42**: 173-178, 2003; Arıkan *et al*., *Amphibia-Reptilia*, **25:** 465-470, 2004; Sevinç *et al*., *Asiatic Herpetol. Res*., **10:** 217-223, 2004; Tosunoğlu *et al*., *Asiatic Herpetol. Res*., **10:** 230-234, 2004; Metin *et al*., *Acta Vet. Brno*., **75:** 49-55, 2006).

Our aim was to describe erythrocyte morphology and measure the erythrocyte and nucleus sizes of some lacertid lizards (*Lacerta trilineata, L. viridis, L. taurica, L. sicula)* which live in Turkey. This study is the first of its kind on Turkish species of those lacertid lizards.

Materials and methods

In this study, 6 (4 ♀, 2 ♂) individuals of *L. trilineata*, 17 (10 ♀, 7 ♂) of *L. viridis*, 5 (2 ♀, 3 ♂) of *L. taurica* and 2 (1 ♀, 1 ♂) of *L. sicula* (Lacertidae) were examined. Three or five blood smears were prepared per individual. The smears were air-dried and stained immediately with Wright's stain technique. On each slide 100 mature erythrocytes and their nuclei were measured by means of an ocular micrometer at a magnification of 1600x (Hartman and Lessler, *Biol. Bull*., **126:** 83-88, 1964).

Results

The erythrocytes or red blood cells (RBC) of the lizards are nucleated, oval cells, and their nuclei are also oval and centrally located like those of the other reptile species. The cytoplasm of mature erythrocytes appeared both light and dark pink and was homogeneous under Wright's stain. The nuclei of mature erythrocytes are chromophilic.

In the present study, the longest erythrocytes were found in *L. sicula*, the shortest ones in *L. trilineata*, the widest ones in *L. taurica* and the narrowest ones in *L. trilineata*. The longest nuclei were recorded in *L. taurica*, the shortest ones in *L. sicula*, the widest ones in *L.viridis* and the narrowest ones in *L. taurica*. Mean erythrocyte and nucleus measurements of examined species with standard deviation were given in Table I.

Discussion

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Reptiles are a heterogeneous group of vertebrates with regard to their blood cell morphology. Normal blood morphology needs to be described for representative species of the 4 major orders of reptiles. Reptilian blood cell investigations carried out by various authors reported that the sizes of erythrocytes vary in members of the 4 orders of reptiles (Hartman and Lessler, *Biol. Bull*., **126:** 83-88, 1964; Szarski and Czopek, *Bull. Acad. Pol. Sci. Cl. II. Ser. Sci. Biol*., **14:** 437-443, 1966; Saint Girons, *Biology of the Reptilia*, Vol. **3**, pp. 73-91, 1970; Canfield and Shea, *Anat. Histol. Embryol*., **17**: 328-342, 1988; Alleman *et al., Am. J. Vet. Res*., **60:** 507-514, 1999; Sevinç *et al*., *Turk. J. Zool*., **24:** 207-209, 2000; Sevinç *et al*., *Asiatic Herpetol. Res*., **10**: 217-223, 2004; Uğurtaş *et al*., *Zool. Stud.*, **42**: 173-178, 2003).

Table I.- Mean erythrocyte dimensions of examined species with standard deviations.

Examined species	EL (µm)	EW (µm)	NL (µm)	NW (µm)
Lacerta sicula	16.96±0.84	8.41±0.65	6.04±0.57	3.02±0.36
L. trilineata	14.57±1.08	8.04±0.60	6.07±0.70	3.04±0.35
L. taurica	14.80±1.01	8.61±0.68	6.36±0.75	3.00±0.38
L. viridis	15.67±1.39	8.13±0.69	6.06±0.74	3.06±0.50

Abbreviations used: EL, erythrocyte length; EW, erythrocyte width; NL, nucleus length; NW, nucleus width.

Within the class Reptilia, the largest erythrocytes are seen in *Sphenedon puctatus*, turtles and crocodilians. Saint Girons (*Biology of the Reptilia*, Vol. **3:** 73-91, 1970) reported erythrocytes and nuclei measurements of *Lacerta agilis* and *L. vivipara*. He measured only erythrocyte and nucleus length. Erythrocyte size of *Lacerta rudis* was measured by Sevinç *et al*. (*Turk. J. Zool*., **24**: 207-209, 2000). Sevinç and Uğurtaş (*Asiatic Herpetol. Res*., **9**: 122-129, 2001) reported erythrocyte and nucleus measurements (both length and width) of *L. rudis bithynica* (=*L. saxicola bithynica*).

In reptiles, lizards have more erythrocytes than snakes, and turtles have the fewest ones. Since the lizards have the smallest erythrocytes of all reptiles, and turtles the largest, there may be an inverse correlation between the number of erythrocytes and their size. This hypothesis was advanced by Ryerson (*J. Ent. Zool*., **41**: 49-55, 1949).

In the present study, erythrocyte morphology and the results of erythrocytes and nuclei sizes are agreement with the other results carried out by Hartman and Lessler (*Biol. Bull*., **126**: 83-88, 1964), Szarski and Czopek (*Bull. Acad. Pol. Sci. Cl. II. Ser. Sci. Biol.*, **14**: 437-443, 1966), Saint Girons (*Biology of the Reptilia*, Vol. **3**, pp. 73-91, 1970), Canfield and Shea (*Anat. Histol. Embryol*., **17**: 328-342, 1988), Alleman *et al*. (*Am. J. Vet. Res*., **60**: 507-514, 1999), Sevinç *et al*. (*Turk. J. Zool*., **24**: 207-209, 2000), Sevinç and Uğurtaş (*Asiatic Herpetol. Res*., **9**: 122-129, 2001), Martinez-Silvestre *et al*. (*Res. Vet. Sci.*, **78**: 127-134, 2005), Carvalho *et al*. (*Comp. Clin. Pathol.*, **15**: 169-174, 2006) and Metin *et al*. (*Acta Vet. Brno.*, **75**: 49-55, 2006).

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CELLULOLYTIC ACTIVITY OF MICROBIAL FLORA OF AGRICULTURAL INSECTS

Abstract.- A grass hopper, *Aiolopus tamulus sevingii*, a bug, red cotton bug, *Dysdercus koningii*, and three beetles *viz.*, red pumpkin beetle, *Aulacophora foeviollis*, blue pumpkin beetle, *Aulacophora attripennis*, and tiger beetle, *Cicindella scutellaris* collected from agricultural fields were screened for cellulolytic microbes. The grass hopper was found to harbour *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Red pumpkin beetle, blue pumpkin beetle and tiger beetle harboured, respectively, *Pseudomonas* spp, *Enterobacter cloacae* and *Staphylococcus* spp., whereas the red cotton bug was found to contain *Bacillus* spp. in their whole body homogenates. All these microorganisms could grow on nutrient agar containing cellulose as carbon source.

Keywords: Cellulase activity, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Psedomonas* spp, *Enterobacter cloacae*, *Staphylococcus* spp.

Cellulose is the major polysaccharide component of plant cell walls and the most abundant renewable energy source on earth. In the biological conversion of cellulose to glucose, at least three distinct types of glycolytic enzymes are involved (Marsden and Gray, *CRC Crit. Rev. Biotech*., **3:** 235-276, 1986). Endoglucanases (endo-1,4-ß-glucanase, EC 3.2.1.4) randomly hydrolyze 1,4-ß bonds of the cellulose chains. Cellobiohydrolases (exo-1,4-ß-glucanase, EC 3.2.1.91) cleave cellobiosyl units from non reducing ends of the cellulose chains. ß-Glucosidases (EC 3.2.1.21) cleave glucosyl units from nonreducing ends of cello-oligosaccharide (Marsden and Gray, *CRC Crit. Rev. Biotech*., **3:** 235-276, 1986). The diverse spectra of cellulases are classified into 12 of the 57 glycosyl hydrolase families based on amino acid sequence similarities (Li *et al.*, *Eukary. Cell*, **2:** 1091-1098, 2003).

Polysaccharide hydrolysis is a key element in insect nutrition. Since the diet of most arthropods comprises primarily plant matter. These polysaccharides (cellulose, hemicellulose and pectin) are resistant to degradation, and the insects themselves do not secrete all of the digestive enzymes to hydrolyze β-linkages in the polymer. Rather, much of the hydrolysis of these polysaccharides is carried out by enzymes produced by the microbes. The microbes help insects in two ways (1) as phytopathogen, by residing on the host plant and producing a number of enzymes and toxins to soft or percolate the leaf surface and make it accessible to insects to attack the cellulose (Andrews and Harris, *Ann. Rev. Phytopath.,* **38:** 145-180, 2000; Beattie and Lindow, *Ann. Rev. Phytopath.*, **33:** 145-172, 1995). (2) as symbiont, by residing in the gut of the insects and producing enzymes for the digestion of cellulose and hemicellulose (Lacy and Lukezic, In: *Plant pathology: Concepts and laboratory exercises,* CRC Press, pp. 41-52, 2003).

The arthropod gut is a differentiated organ harboring a complex biotope comprising both resident and transient members from protozoan, bacterial, and archaeal genera (Idowu and Edema, *Global J. Pure appl. Sci***., 8:** 447-454, 2002). In the digestive tracts of lower termites, cellulose seems to be synergistically degraded by flagellates, bacteria, and yeasts as well as by the termite's own cellulases (Li *et al*., *Eukary. Cell*, **2:** 1091-1098, 2003).

A similar situation has been observed in the wood-roach *Cryptocercus punctulatus,* which depends on cellulase generated by protozoan present in the hindgut (Huub *et al*., *Appl. environ. Micrbiol*., **60:** 1822-1826, 1994). The microbial flora of the gut regions and gut contents of an insect could provide a proper understanding of the physiological processes involved in the hydrolysis of cellulose.

A number of microbes comprising of 30 genera has been identified as phytopathogens including *Agrobacterium, Xanthomonas, Erwinia* (Lacy and Lukezic, In: *Plant pathology: Concepts and laboratory exercises*, CRC Press, pp. 41-52, 2003). It has not been identified that the plant pathogens are human pathogen or not. Phytopathogens are able to colonize within the plant tissue and produce a number of enzymes and toxins which weaken the plant tissue and make it susceptible to pest attack (Denny, *Annu. Rev. Phytopathol.*, **33**: 173-197, 1995; Hahn, *Annu. Rev. Phytopathol.*, **34**: 387-412, 1996). Not much attention has been given to phytopathogen residing on the host plants for cellulose hydrolysis. Here we report on the screening, isolation and identification of cellulolytic microbial flora from the homogenate of local insect species *viz.*, *Aiolopus tamulus sevingii, Aulacophora foeviollis, Dysdercus koningii, Aulacophora attripennis* and *Cicindella scutellaris.* We have previously reported the presence of cellulase activity in the total homogenate of insects *A. foeviollis, D koningii, A. attripennis* and *C. scutellaris* (Sami and Shakoori, *Pakistan J. Zool.*, **38:** 337-340, 2006).

Materials and methods

All chemicals used were of analytical grade.

Insects were collected from Agricultural fields of Lahore District. All insects were collected in separate sterilized bottles and then stored at -20°C. Prof. Dr Shamshad Akbar, Department of Zoology, Govt. College University, Lahore identified the insects. After sample collection and identification, insects were subjected to preliminary screening to detect cellulase activity.

A weighed quantity of insects was taken from stored sample and homogenized in 0.5 M Tris: HCl buffer pH 8.5 and centrifuged at 10,000Xg for 10 minutes. The supernatant was stored in 1 ml aliquot

in eppendorf tubes and used as a source of enzyme.

The screening procedure was based on the method as previously described (Sami and Shakoori, *Pakistan J. Zool.*, **38:** 337-340, 2006)

The insects were ground and streaked on media plates (nutrient agar + CMC) for microbial isolation. The plates were incubated at 37°C for 24 hours. Different microbial colonies appeared on the media plates after 24 hours. Each microbial colony was streaked on three different media (nutrient agar + CMC, nutrient agar + glucose and nutrient agar + Avicel) for pure culture.

All bacterial isolates were maintained on Nutrient agar slants as well as in glycerol and identified according to Benson (*Microbiological application, complete version: Laboratory manual in general microbiology.* Wan C. Brown Publishers, Dubuque, Melbourne, Oxford, 1994).

Results and discussion

A. tamulus sevingii was also able to hydrolyse cellulose when tested on carboxy methyl cellulose agar plate (Fig. 1).

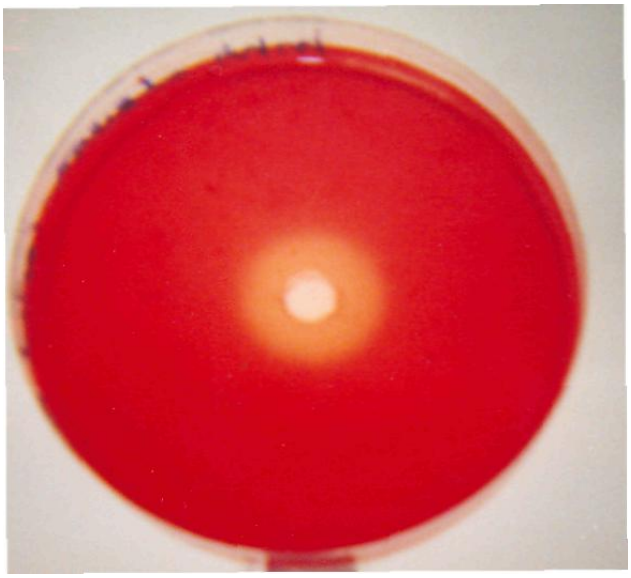

Fig. 1. Screening of cellulase activity of whole body homogenate of a grass hopper, *Aiolopus tamulus sevingii* on CMC-agar plate.

This study was undertaken to isolate the bacterial strains present in the homogenate of the insects capable of hydrolysing cellulose. These insects live in the fields or in surroundings of the host plants and carry the microbes sticking to their host plant as phytopathogen. All the insects studied in this report contained cellulose in their homogenate (Sami and Shakoori, *Pakistan J. Zool.*, **38**: 337-340, 2006) including A. tamulus (Fig. 1). A destructive pest of cucurbit plant red pumpkin beetle *A. foeviollis* had *Pseudomonas* spp. in its homogenate. *Pseudomonas* spp. is reported to be a phytopathopathogen with ability to produce a number of enzymes including pectin lyases and toxins which cause the membrane to leak. *Pseudomonas* spp. is also associated with angular leaf spot disease of cucurbits family, which is also associated with the pest *A. foeviollis* (Lacy and Lukezic, In: *Plant pathology: Concepts and laboratory exercises*, CRC Press, pp. 41-52, 2003). At present there is no relationship reported between the *A. foeviollis* and *Pseudomonas* spp. for angular leaf spot disease of plants. The common grass hopper, *A. tamulus sevingii*, had *A. baumannii* and *K. pneumoniae* in its homogenate. *Klebsiella* has been previously reported as plant pathogens (Lacy and Lukezic, In: *Plant pathology: Concepts and laboratory exercises*, CRC Press, pp. 41-52, 2003), while *A. baumannii* has not been previously reported. In this study two predators were included, red cotton bug, *D. koenigii* had *Bacillus* spp. in its homogenate, while tiger beetle *C. scutellaris* had *Staphylococcus* spp. For blue pumpkin beetle *A. attripennis* a bacterial strain *E. cloacae* was identified. Enterobacter has previously identified as a plant pathogen (Andrews and Harris, *Annu. Rev. Phytpathol.*, **38**: 145-180, 2000). Future studies could be concentrated on the cellulase and hemicellulase production of the phytomicrobes.

Table I.- Shows the isolation of different bacterial strain able to hydrolyse the cellulose from insects which were collected from New Campus, University of the Punjab, Lahore.

Insect (common name)	Zoological name	Bacteria isolated
Grass hopper	*Aiolopus tamulus sevingii*	*Acinetobacter baumannii; Klebsiella pneumoniae*
Red pumpkin beetle	*Aulacophora foeviollis*	*Pseudomonas* spp.
Red cotton bug	*Dysdercus koenigii*	*Bacillus* spp.
Blue pumpkin	*Aulacophora*	*Enterobacter*

beetle	*attripennis*	*cloacae*
Tiger beetle	*Cicindella scutellaris*	*Staphylococcus* spp.

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FIELD EVALUATION OF COUMATETRALYL BAIT AGAINST INDIAN CRESTED PORCUPINE, *HYSTRIX INDICA* KERR

Abstract.- The field efficacy of coumatetralyl bait (0.0375%) was studied against *Hystrix indica* on a floriculture farm, where Gladiolus and Dutch Irish were being severely damaged. The bait was placed underneath the bait stations, established either near the porcupine dens or at entry points along the fence line of the farm. Bait consumption increased up to 7th day, when it steadily decreased by the 14th day, reaching zero level on the 15th day. As a result of baiting, dead porcupine showed symptoms of anticoagulant poisoning. Analysis of post-treatment porcupine activity showed no signs of activity, indicating 100% reduction of porcupine population.

Key words: Bait station, anticoagulant, baiting, efficacy, management

The Indian crested porcupine, *Hystrix indica*, an Old World porcupine species, is a serious vertebrate deteriogen of economic importance in agriculture and forestry systems (Khan *et al*., *Int. Biodet. Biodeg*., **45**: 143-149, 2000). It is abundant and widely distributed in all the agro-ecological zones of Pakistan (Roberts, *Mammals of Pakistan*, pp. 525, Oxford University Press, Karachi, 1997). *H. indica* is, also, a generalist forager that exploits a wide variety of cultivated and wild plants, and consumes above ground as well as sub-surface plant tissues (Gutterman, *J. Arid Environ*., **5**: 261-268,1982). Khan *et al*. (*Int. Biodet. Biodeg*., **45:** 143-149, 2000) recorded five species of grasses which were severely damaged by porcupine digging at the Karluwala desert range, district Bahkhar, Punjab, Pakistan. Similar damage was observed to saffron (*Crocus sativa)* plantation at Mustang, Balochistan (Pakistan). The study site *i.e*., Allied Floriculture Farm, Islamabad, has a previous history of porcupine damage to Gladiolus and Dutch Iris plantation, cultivated for commercial purpose. The owner of the farm reported 30-70% damage to bulbs of these plants. In view of severe porcupine damage to the flowering plants and sufficient porcupine activity on the farm, field efficacy of 0.0375% coumatetralyl bait was assessed.

Materials and methods

The study site, Allied Floriculture Farm is located in H-9/3, Islamabad, and having properly fenced boundary. The bait station (Fig.1) was properly fabricated from 205 liters capacity steel drum, cut into two pieces along the length into half round culverts (Neitro, 4th Vert. Pest Conf., Univ. Calif. Davis, pp. 98-100, 1970). Eight bait stations were established near the porcupine entry points along the fence and two in front of the porcupine dens. The bait stations were camouflaged with green vegetation. Coumatetralyl (0.0375%) bait was prepared (w/w) from whole maize and molasses in the ratio of 15:2.5:2.5. The baiting was conducted for two weeks. For first three days 500 g of bait was placed under each bait station in an earthen bowl (30 cm diameter and 6 cm deep) and for the next five days 1kg bait was used. Bait consumption was recorded daily and weighed by Pesola spring balance near to 0.1 g accuracy. Pre-post-treatment porcupine activity observations were made by laying dust patches (1x1 m) across the porcupine trails and in front of porcupine dens.

Results and discussion

Data collected for two weeks showed that pattern of coumatetralyl (0.0375%) bait consumption (Fig.2) by porcupine was very much similar to that of anticoagulants by field rats (Hussain and Prescot, *Pakistan J. Zool*., **38**: 355-

360, 2006). Increase in consumption of bait by porcupine gradually increased reaching the maximum value on the 7th day, when it steadily decreased reaching to zero level by the 15th day. Analysis of cumulative bait consumption data of 10 bait stations for 14 days did not show much consumption variations within the feeding days. The pattern of variation was characteristic of anticoagulant bait consumption (Hussain and Prescot, *Pakistan J. Zool*., **38**: 355-360, 2006).

Fig. 1. Porcupine bait station.

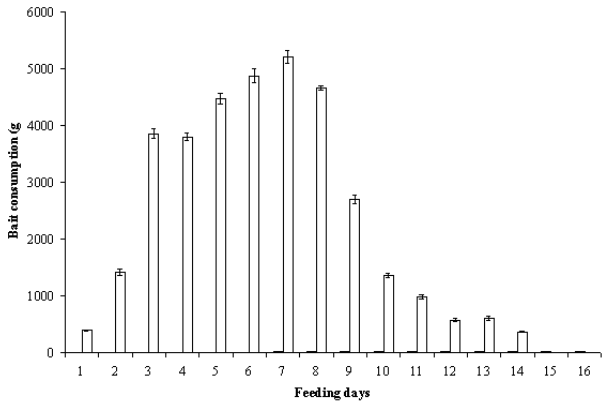

Fig. 2. Pattern of coumatetralyl bait (0.0375%) consumption by *Hystrix indica* using protected bait station

Observations were made on fresh scattered fecal pellets to know bait consumption by porcupines and development of pathological symptoms. On the 5th and 6th day of bait placement loose fecal pellets of bluish colour, the warning dye added to the master mix (0.75% coumatetralyl) were seen. This indicated that bait offered at bait stations is being consumed by porcupines and that the pathological symptoms have started to appear among the individuals of the porcupine population on the farm. On the 9th day of baiting one dead porcupine (approximately 15-16 Kg) was found near bait station No.7, which died during the previous night, or most probably, early morning. Bleeding from nose and eyes was noted which clearly indicated anticoagulant symptoms of death. After termination of baiting on the 15th day search was made of carcasses throughout the farm area. As a result two dead porcupines were located in thick tall grass fields. No non-target bird species was found, though some partridge population was present in the treatment area. The colour of the bait and grain size prevented the birds from eating the bait. All the dens located on the farm and entry points along the fence line were tracked for two nights. No positive signs of porcupine presence were recorded on track patches, meaning that the resident as well as the visiting porcupine populations had reduced to almost zero level.

The use of anticoagulant baits against Indian crested porcupine has inadequately been studied. Ahmad *et al.* (*Pakistan J. nat. Hist. Wild*., **2**:19-23, 2003) used 0.005% brodifacoum wax blocks, but did not record daily consumption. Similarly, Khan *et al.* (*Pak. J.scient. indust. Res*., **49**: 418-422, 2007) used one Kg of whole maize grain bait of coumatetralyl (0.0375%) per den but did not record daily bait consumption. However, results of both the studies showed that anticoagulants are highly effective against *H. indica*. The present study, therefore, is first of its kind, wherein daily bait consumption was recorded and field efficacy was properly assessed by employing pre- and post-treatment tracking observations. The bait station used in this study protected the bait from the weather, killing of non-target animals and tampering. Also, the bait station is durable, can be camouflaged, safe, and can easily be constructed. The study showed that coumatetralyl (0.0375%) baiting, using the bait station, was highly effective and economical in controlling porcupines. The significant reduction in damage to flowering plants was also indicative of this fact. Further studies with second generation anticoagulant baits are suggested.

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