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Identification of Prey Remnants in the Gut of Spiders by Polyacrylamide Gel Electrophoresis (PAGE) of Esterase Isozymes

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Abstract: A controlled feeding laboratory experiment was conducted in which spider species, *Pholcus phalangioides, Plexippus paykulli*, *Oxyopes shweta, Cyrtophora citricola* and *Marpissa dhakuriensis* were used as predators while *Musca domestica, Sitophilus oryzae*, *Bactocera cucurbitae and Pyrilla perpusilla* were used as prey species. Predators fed with and without prey and only preys were macerated and subjected to non denaturing poly acrylamide gel electrophoresis (PAGE). Isozyme (esterases) banding patterns of predators (spiders) and preys were obtained and prey proteins were detected in the gut of predators. This method is very helpful in determining the predatory efficiency of spiders.

Key word: Spiders · Prey remnant · Gut · Isozyme · Polyacrylamide gel electrophoresis (PAGE)

INTRODUCTION

Predation studies are of great ecological, evolutionary and behavioral interest. Recent trends in agriculture towards reduce pesticide use and ecological sustainability has lead to enhance the interest in spiders as biological controlling agent of insect pests. Spiders not only reduce the pest densities to below economical threshold levels but also stabilize pest densities over the time. They have been used as the natural enemies of pest in several key crops and are gaining importance throughout the world as successful agents of biological control due to their ability of intensive predation. It is well known that under crop conditions, spiders are important enemies of aphids, mites and Lepidopteron larvae and eggs [1]. As such, spiders have become a key component of the integrated pest management (IPM) programmes.

Primary reason of this study is to determine the role of spiders in suppressing the pest populations. It is an important phenomenon for controlling the insect pest of various crops and can be studied by different means like direct observation, experimental field manipulation, laboratory feeding studies and gut analysis [2]. Among these phenomena gut analysis of spiders is of great importance. Regarding gut analysis various methods are used to explore the predation including the application of radionuclides [3], chromatography [4], nucleic acid probes [5], serological studies [6] and electrophoretic techniques [7]. Polymerase chain reaction (PCR) technique has also been applied for the gut analysis of aphid, arthropods etc. [8, 9]. Electrophoresis separates proteins on the bases of charge and size differences in an electrical field. Differences in charge and size commonly occur among isozymes, the proteins catalyzing the same reaction but different structural properties. To study the predation of spiders by gut analysis the electrophiresis technique has been utilized by many investigators [7, 10]. Polyacrylamide gel electrophoresis was used to study gut analyusis of spiders in order to explore the phenomenon of predation [11, 12]. Electrophoretic analysis of predator is relatively inexpensive and simple biochemical technique requiring no sophisticated equipment. The chemicals required are comparatively inexpensive. The method provides quick results and large sample volumes can be processed within a short period of time. Moreover, prey consumption can be detected even 48 h after predation. Hence, these techniques are particularly suited for the analysis of the prey spectrum [13]. In this recent experiment poly acrylamide gel electrophiresis (PAGE) technique has been used to study the phenomenon of predation by the gut analysis of spiders. This technique is a biochemical simple and inexpensive requiring no

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sophisticated equipments. The objectives of the present study were to determine the effectiveness of isozyme aalysis in the detection of prey remnants in spider gut and the evaluation of the effectiveness of spiders this method to study as potential predators of insect pests.

MATERIALS AND METHODS

Collection and Identification of Spider Species: Fields of nuclear institute for agriculture and biology (NIAB) Faisalabad were selected for the collection of spiders. Collection was made in the morning or evening and only manual collection was done. For this purpose, spiders were captured from their webs and fields with the help of glass jars and then brought to laboratory for further identification. The collected spiders were identified to species level using relevant available literature. *Pholcus phalangioides (fuesslin)*, *Plexippus paykull (audouin)i*, *Oxyopes shweta, Cyrtophora citricola* and *Marpissa dhakuriensis* were found to be the commonly occurring species. Before use for gut analysis studies, the spiders were kept in the laboratory under starvation.

Collection and Identification of Preys Species: Specimens of common house fly *Musca domestica L.* (Muscidae: Diptera) were collected from NIAB colony with the help of insect net and larvae of rice weevil [*Sitophilus oryzae* Linnaeus (Curculionidae: Coleoptera)] from the rice grain store. Larvae of melon fruit fly [*Bactocera cucurbitae* Coq. (Tephritidae: Diptera)] were captured from the rotten vegetables. Nymphs and adults of Sugarcane leafhopper [*Pyrilla perpusilla* Walker; (Lophopidae: Homoptera)] were collected from the sugar cane fields of NIAB. The collection was made with the help of glass jars.

Treatments and Maceration: To each individual starved spider different prey species were given separately and after an hour of feeding predators were macerated in Eppendorf tubes containing phosphate buffer of pH 7.5. For each treatment there were three samples one sample of prey, another sample of predator with empty gut and third sample of predator to which prey was given. After half a minute of maceration, with glass rod, the samples were centrifuged for 10 minutes at 14,000 rpm and supernatants were collected for isozyme analysis.

Analysis of Gut Contents by Polyacrylamide Gel Electrophoresis (PAGE): For electrophoretic separation of proteins polyacrylamide gel electrophoresis (PAGE) with non dissociating discontinuous buffer system was used for isozyme profiling as described by Laemmli (1970). Gel sandwich was fixed in dual vertical gel electrophoresis unit [14]. Gels were loaded with 20-25 micro liter sample and electrophoresed at constant voltage 175V. Gels were histochemically stained for esterase activity following the method of Ainsworth [15].

RESULTS

Figure 1 presents the results of gel electrophoresis of the prey (*M. domestica*) and two predators i.e., *P. phalangioides* and *P. paykulli*. One strong band of esterase activity can be observed for the prey *M. domestica* (lane-1) while two predators, *P. phalangioides* (Lane-2) and *P. paykulli* (Lane-4) show five and seven bands, respectively, in their esterase profiles. Prey specific esterase activity was observed in the gut contents of both the spiders one hour after feeding on the given prey (Lane 3 and 5) and is marked with asterisk (*) on the activity gel. The two species of spiders can also be identified or distinguished on the basis of their esterase profiles. A total of 10 activity zones were observed in the profiles of *P. phalangioides* and *P. paykulli* out of which 6 were polymorphic. Zymogram of the activity gel is presented with the activity gel for easy identification of the bands.

The results of two predators *P. paykulli* and *C. citricola* before and after feeding on prey (*P. prepusilla*) are presented in the Figure 2. Four esterase bands (lane 1) were present in the profile of *P. perpusilla* (prey) two of which were thick bands of higher intensity while two were thin bands of low intensity. In the control profiles of *P. paykulli* (predator) six bands of high and one of low intensity can be observed (lane-2). Lane 4 shows the esterases banding pattern of *C. citricola* (predator) before feeding on prey. Five low intensity bands can be seen in the activity gel. Lane 3 showing the isozyme profiles of *P. paykulli* after feeding on *P. perpusilla* prey and one specific band (with asterisk) could be detected confirming the presence of *P. perpusilla* proteins in the predator gut. Similarly, by comparing the profiles of starved and fed *C. citricola* the same band can be observed after feeding (lane 5, band with asterisk). A total of twelve activity zones were observed in the profiles of two predators (*P. paykulli* and *C. citricola*) and eight of this showed polymorphism.

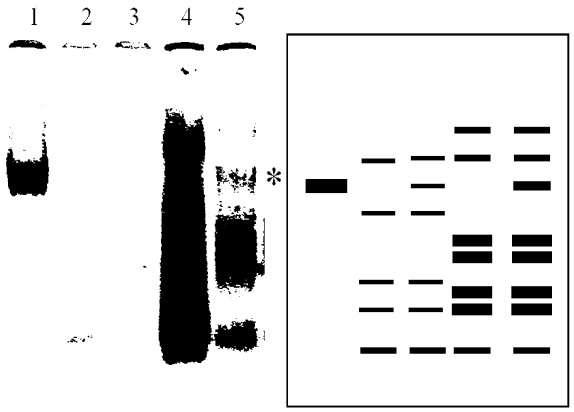

Fig. 1: Lane-1. *Musca domestica* (prey), Lane-2 *Pholcus Phalangioides* (without prey), Lane-3 *Pholcus Phalangioides* (with prey), Lane-4 *Plexippus paykulli* (without prey), Lane-5 *Plexippus paykulli* (with prey).

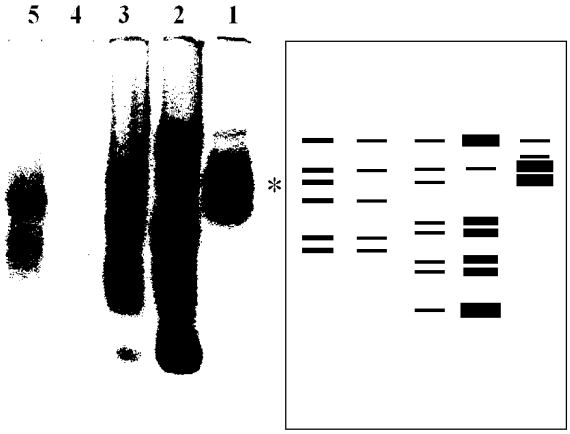

Fig. 2: Lane-1 *Pyrilla perpusilla* (prey), Lane-2 *Plexippus paykulli (*without prey), Lane-3 *Plexippus paykulli*(with prey), Lane-4 *Cyrtophoracitricola*(without prey), Lane-5 *Cyrtophora citricola* (with prey).

Figure 3 illustrates the esterase activity-banding pattern for the *P. perpusilla* prey profile show four bands, two major darkly stained bands and two faintly stained minor bands. One major and one minor band were monomorphic to the predator (*C. citricola*) profiles while the other two were polymorphic. Esterase profiles of predator (*C. citricola*) showed four major and one minor band with three fast moving bands polymorphic to the prey (Lane 2). Lane 3 shows the predator (*C. citricola*) profiles after feeding on prey (Nymph *P. perpusilla*). A prey specific band (marked with asterisk) in the profile indicated the presence of prey proteins in the gut of

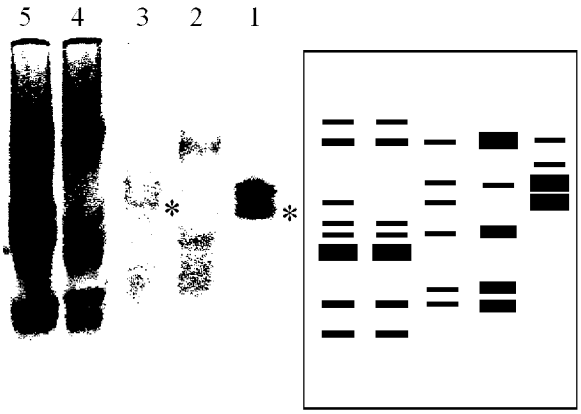

Fig. 3: Lane-1 Pyrilla perpusilla nymph (prey), Lane-2 Cyrtophora citricola (without prey), Lane-3 Cyrtophora citricola(With prey), Lane-4 Marpissa dhakuriensis(without prey), Lane-5Marpissa dhakuriensis (with prey)

C. citricola. Lane 4 and 5 show esterase profiles of *M. dhakuriensis* spiders before and after feeding on nymph *P. perpusilla* respectively. The two lanes can be distinguished on the basis of one prey specific polymorphic band (Lane 5 marked with asterisk). Zymogram, representing the activity gel shows a total of 11 different bands resulting from the variation in their mobility. The band No. 7, a prey specific band absent in the profiles of predators, appeared only after the feeding of predators on the prey. The two predators showed a total of twelve bands in both their profiles; six of these bands were polymorphic between the two species.

Eight distinct bands were observed in the esterase profiles of *O. shweta* while three bands could be observed for the *S. oryzae* and five activity zones for *B. cucurbitae*. One slow moving band of *S. oryzae* shows mobility similar to one of the bands in the predator profiles while two other bands were polymorphic in nature. For *B. cucurbitae* larvae, out of five bands three were monomorphic while two were polymorphic to *O. shweta* profiles. A fast moving *S. oryzae* larva band was also present in predator profiles fed on *S. oryzae* larvae. Similarly, a band specific to *B. cucurbitae* larva can also be seen in the gut profiles of *O. shweta* after feeding on *B. cucurbitae.* The two preys, S. *oryzae* and *B. cucurbitae*, showed total of eight bands in both their profiles while six of these bands were polymorphic.

In case of *P. paykulli* fed on *S. oryzae* and *B. cucurbitae* two out of three *S. oryzae* bands were polymorphic to prey profiles and one of those was also

present in the profile of *P. paykulli* fed on *S. oryzae*. Three polymorphic bands were identified in the prey *B. cucurbitae* profile from that of its predator but only the slowest mobility activity zone was stained in the *P. paykulli* profiles after feeding on the prey.

DISCUSSION

Plexippus paykulli, Oxyopes shweta, Marpissa dhakuriensis, Pholcus phalangioides and *Cyrtophora citricola* were the common predators identified in the present studies. Earlier, *Plexippus paykulli, Marpissa dhakuriensis* have been used for the taxonomic study of the spider fauna of Lahore [16] and are easily available for predation study. Likewise, *Musca domestica, Sitophilus oryzae, Bactocera cucurbitae* and *Pyrilla perpusilla* were found to be the common prey species and were used in the predator prey studies reported here. Identifying and quantifying prey remains in the gut are the first steps in determining spider predation rates [17]. Specific esterases patterns allow the identification of prey remnants inside the predator,s gut [18].

Several esterases bands were obtained for prey larvae *Cytophilus oryzae*, *Bactocera cucurbitae* and *Pyrilla perpusilla* which are very similar to the banding patterns obtained by Dicke and Dejong [10]. But for the prey Musca domestica only one esterase band was obtained and this observation is very similar to that reported by Murray and Solomon [7].

Although previous reports have provided the possibilities of detecting prey remnants inside predator gut by isozyme analysis but the proportion of the prey proteins detected by means of PAGE was rather limited. In the present research samples were taken after the continuous feeding for 1-2 hour to increase the possibility of detecting all polymorphic bands of prey species in the predator profiles. Although mostly more than one polymorphic esterase bands were observed between the prey and predator species, but in all cases only one polymorphic band could be identified into predator profiles after controlled feeding on a specific prey.

Although this study was performed under controlled conditions for predation but the results suggest that this method can also be utilized under field conditions to better understand the role of spiders in biological control of insect pests. Based on our results all the prey and predator species were distinguished by only one isozyme system. Therefore, it is possible to generate reference profiles of predators and preys that can be used when studying predation in field conditions. Different isozyme systems may also be used for comprehensive profiles of economically important species that may further provide information about the population structure of preys and predators. Further studies are needed using a mixture of preys and establishing isozyme systems to differentiate their remnants in the spider gut. Extensive studies on these lines may help determine the relative population of different prey types and/or preference of predator for one or more types. This approach will help to establish the population dynamics of different pest species.

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