Short Communications

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Susceptibility of Wolf Spider, *Lycosa terrestris* (Araneae: Lycosidae) to Chlorpyrifos

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Abstract.- The present study was designed to check the effect of chlorpyrifos (Lorsban) on *Lycosa terrestris*, the most dominant ground spider in the agroecosystem of Punjab, Pakistan. For this purpose topical and residual bioassay tests were performed. Probate analysis showed that this insecticide is highly toxic for both sexes. The calculated LD_{50} values were less than field dose. Results of residual bioassays showed that this insecticide is highly toxic to spiders even after one month of its exposure. Negative effects of this insecticide not only reduces the biological control potential of *L. terrestris* but also poses a serious threat to other natural predators of agroecosystems.

Key words: Agroecosystem, residual toxicity, ground spiders.

Spiders are the most abundant natural predators of insects in terrestrial ecosystems and consume large number of prey without damaging the plants (Lang *et al.*, 1999; Hanna *et al.*, 2003). Many studies have demonstrated that spiders can significantly reduce prey densities in agricultural fields (Symondson *et al.*, 2002; Pearce and Zalucki, 2006; Schmidt *et al.*, 2009). Agricultural fields that are frequently sprayed with pesticides often have low spider population (Amalin *et al.*, 2000).

Cyhexatin, flubenzimine, dicofol and azocyclotin) and chlorinated hydrocarbons (endosulfan) followd by pyrethroids, organophosphates and carbamates have been reported to be most toxic the hunting spiders and web-builders (Mansour and Nentwig, 1988). Some insecticides, specificially deltamethrin, dimethoate and phosalone do not cause substantial spider mortality but can greatly disturb spider predatory activity over a variable period, depending on the insecticide and the spider species involved (Cocquempot *et al.*, 1991). In addition to causing mortality, pyrethroid (specifically Fenvalerate and Lambda-Cyhalothrin), when applied at lower doses, can inhibit spiderling emergence from cocoons and delay web building activity (Dinter and Poehling, 1995).

Present study was designed to check the susceptibility of both sexes of wolf spider, *Lycosa terrestris* (Araneae: Lycosidae) to commonly applied insecticide. Chlorpyrifos by topical and residual exposure methods.

Material and methods

The male and female specimens were collected by hand picking and handy-vacuum (SIEMENS VK 20C01) from the edges of a guava orchard situated on Shahadra Road Lahore, 5 Km from Sagian Bridge from April through November, 2008. To check the effects of Chlorpyrifos on spiders, topical and residual bioassay tests were performed. Each experiment was replicated thrice. This insecticide is commonly used on grains, cotton fields, fruits, nuts, vegetable crops as well as on lawns and ornamental plants in the study area.

For topical application four concentrations of chlorpyrifos in acetone *viz.* 100, 200, 300 and 400 ng *a.i.* per individuals were tested. Before application of insecticide, spiders were anesthetized by brief exposure to CO_2 gas (for 20 seconds). The insecticide was applied (0.5 µl) topically on the dorsum of each spider using a micropipette. Response of organism was recorded as unaffected (showing normal behaviour), affected (showing irregular pattern of movement), paralyzed (completely inmobilized) and dead (totally non-responsive) for each dose after 1, 2, 4, 8, 16 and 24 hours of exposure.

For dry residue testing, half inch thick layer of soil was taken in experimental pots (10 inch diameter × 5 inch high) and dried in sun before spray. Insecticide was sprayed at field rate on the pots by using Knapsack hand sprayer (THS-119428). Control pots were sprayed only with acetone. Ten male and ten female spiders were

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released in the treated pots after 1, 2, 3, 5, 7, 10, 15, 20, and 30 days of spray and result was noted at 1, 2, 4, 8, 16, 24, 48, 78, 96 and 120 hours after the release. The spiders were fed with larvae of wild-type *Drosophilla* cultured in the laboratory. Mortality rate and other effects of insecticides were assessed daily by the examination of individuals.

Statistical analysis

For both experiments mortality was calculated for each concentration of insecticide. Results obtained from the dose response series evaluation after 8th hour for topical exposure and 48th hour for residual toxicity were subjected to Probate analysis to determine the LD_{50} and LD_{95}. Dosages were expressed as nano-gram of active ingredients per treated individual (ng a.i./ individual).

Results

Results showed that the susceptibility of chlorpyrifos is positively associated with amount of insecticide applied and duration of exposure. Topical bioassay test showed that field rate caused 100% mortality after 16 h in male individuals and after 24 h in female individuals. Double field rate caused 100% mortality after 4 h in male and after 8 h in females. The summary of Probate analysis at 8th hour assessment data is given in Table I. Resistance ratio shows that females are twice resistant than males. The percentage of mortality increases with time for all doses. The summary of Probit analysis at 48th h assessment data is represented in Table II. Results showed and chlorpyrifos remains active till 27 days for the spider specimens.

Table I.- Estimated LD_{50} and LD_{95} for Chlorpyrifos (ng a.i./individual) calculated at 8th h of application of both sexes of *L. terristris* for topical exposure method.

Sex	LD %	Dose (Confidence limit)
Male	50	80.42 (48.01-101.18)
	95	222.69 (198.59-262.39)
Female	50	180.93 (167.16-193.95)
	95	305.32 (284.80-332.76)

Table II.- Estimated LT_{50} and LT_{95} of residual field rate of Chlorpyrifos calculated at 48th h of both sexes of *L. terristris* .

Sex	LD %	Dose (Confidence limit)
Male	50	24.87 (23.16-26.92)
	95	6.95 (4.62-8.90)
Female	50	27.92 (25.74-90.69)
	95	7.48 (7.74-9.65)

Discussion

In the present study males had high susceptibility than females, although the ratio of susceptibility varies in all treatments of insecticide. The high variability was consistent with the result of Baatrup and Babylay (1993). A number of studies have reported differences in susceptibility for both sexes (Dinter and Poehling, 1995). Higher mortality in males in the present study is in accordance to Peterson (2002). High mortality in males might be due to application of high dose of insecticide relative to their body size. However, Nielson *et al.* (1999) reported no size effect on the susceptibility of *Pardosa amentata* caught from the field population.

Variations in the susceptibility levels among individuals were also recorded during the study. This might be due to the reason that spiders were collected from the field and such individuals have an unknown feeding history and determining level of starvation is very difficult. Although individuals in the present experiment were fed to satiated level prior to experimentation, variations may still be present in the degree of satiation among individuals. Peterson (2002) found starved *P. amentata*, or individuals fed on low quality prey to be more susceptible to the insecticide.

The post exposure after 24 h showed that reduction in mortality was very steep. The difference in mortality may be due to difference in the interaction of insecticide with the substrate and environment in the field. The high mortality in *L. terrestris* at field rate suggested that toxic impact of this product would be very high. Affected individuals were either paralyzed or showed un-coordinated walking. This can result in exposure to predators in actual field conditions. So the death rate

would be higher in the field than in the laboratory. Along with spiders, food will also be exposed to insecticide and will cause reduction in the population of spiders.

Long half-life of chlorpyrifos (Anonymous, 2008) suggested that this insecticide can exist for long time in the soil. Laboratory experiments also showed that chlorpyrifos remains active till 27 days for the specimens. In the field, chlorpyrifos may cause reduction in the spider density as well as diversity. Due to high toxicity of chlorpyrifos reestablishment of spider density and diversity may take longer period (even months).

It is concluded from the present work that use of chlorpyrifos is a major threat to the spider fauna in the studied area. So its use should be minimized and alternative measures should be used to control the pests in the orchards and agricultural fields. The current study also highlights the need to investigate the impact of other pesticides which are commonly used on the spider fauna of the study area in order to gain a more realistic view of what may occur in treated orchards and agricultural crops. Furthermore, field based assessments are required to provide the most reliable results to be extrapolated into real environmental situations.

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Occurrence of Whitefly, *Bemisia tabaci* (Genn.) in Chili, *Capsicum frutescens* L., at Multan, Pakistan

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Abstract.- The research was conducted to study the occurrence of whitefly, *Bemisia tabaci* (Genn.), on chili, *Capsicum frutescens* L.**,** in Multan, Punjab (Pakistan). In 2007 five chili varieties were planted whereas in 2008 one variety was planted on three different dates. *B. tabaci* adults were recorded per plant. Whitefly was active during May in both years and did not exceed two adults per plant.

Key words: Whitefly, chilies, Pakistan

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Chilies, *Capsicum frutescens* L., are ingredients of the daily diet of people of Pakistan, India and other Asian countries (Sanyal *et al.*, 2008). They are used as vegetables and as spices for improving the flavour of foods. Green chilies are also consumed; these are rich in vitamin A and C and seeds contain traces of starch (Muthukrishnan *et al*., 1986). Literature reports different results about the pest status of whitefly, *B. tabaci* (Genn.), and other insect pests of chilies from different parts of the world. Chilies had been reported as an alternate host of whitefly, *B. tabaci* in 1930s in Pakistan (Hussain and Trehan, 1933). It has been also argued recently that chilies are important sources of *B. tabaci* that moves into cotton (Attique *et al.*, 2003; Rafiq *et al.*, 2008) in cotton growing areas of Pakistan.

The Multan district of Punjab, Pakistan has an arid climate and receives mean annual rainfall of about 125mm. The winter season extends from November to February. There is a short spring during March (Amer *et al*., 2009). Chilies are sown only once in a year in Multan. Chilies are sown as Kharif crop (summer crop) and normally are transplanted from nursery to field in mid February (Iqbal, 2009). There is no judicious application of insecticides in the developing countries. There are no reports in the literature of detailed research on insect pests, their seasonal activity and management on chilies from Multan. The present study was undertaken to determine the occurrence of *B. tabaci* on different genotypes of chilies and on crops planted at different dates at Multan.

Materials and methods

Experiments were conducted at the experimental farm of the University College of Agriculture, Bahauddin Zakariya University, Multan. During 2007 seeds of five genotypes *viz.*, CV-7, Nepal-S, NARC-4, CV-4 and Nepal-L were obtained from Institute of Horticulture, National Agricultural Research Centre (NARC), Islamabad. Plastic pots (45x45x15 cm size) were filled with fine soil and seeds were sown in these pots. Seed of each variety was sown into a separate pot on February 4, 2007. Pots were placed in a room furnished with electric heater. After two weeks pots were put outside to acclimatize the plants to the open environment. These nursery plants were transplanted into the field on March 15, 2007. Experiment was laid out in a randomized complete block (RCB) design with three replications. Plants were transplanted on a ridge of 4 m length. Distance between ridges was 45 cm and the distance between plants was 30 cm. Each treatment consisted of three ridges. Treatments and replications were 0.5m and 0.9 m apart, respectively. Crop was irrigated when needed. Weeds were manually removed from the crop by hoeing.

The 2008 experiment was also a randomized complete block (RCB) design. Nursery plants of a local variety, Tattapuri, were purchased from the market for each transplanting date and were transplanted at three different sowing dates *i.e*., 3rd, 11th and 18th of March, 2008.

Beds were prepared and chili nursery plants were transplanted on both sides of beds. Each bed was 12 m long and 30 cm wide. Distance between beds was 45 cm and plant to plant distance was 30 cm. There were three replications in each sowing date. Each replication consisted of four beds. Replicates were 0.9 m apart. Uniform practices were followed in both the trials in each year.

In both years the chili crops were observed and *B. tabaci* numbers recorded from transplanting until the end of the growing season. Whitefly numbers were recorded early in the morning from five plants selected randomly from each plot in 2007. For this purpose plants were gently turned on opposite side of sun to count whitefly adults, as these are usually present on lower side of the leaves. Whiteflies were counted from the whole plants.

During 2008 numbers of whiteflies were recorded from 15 plants from each sowing date. Five plants for this purpose were randomly selected from each replicate of a sowing date. Similar method was employed for recording whitefly adults as described for 2007. Numbers of *B. tabaci* were recorded three times in each year, starting from 1st May and 7th May in 2007 and 2008, respectively.

Numbers of whitefly were converted to mean density per plant. Data on mean population were analyzed using Analysis of Variance (ANOVA) and differences among means were separated by calculating Least Significant Difference (LSD) test

(at P= 0.05) (Steel and Torrie, 1980) by computer software MSTATC (MSU, 1982).

Results and discussion

Numbers of *B. tabaci* per plant were non-significantly different on all the genotypes during 2007. Numbers were less than two per plant on all genotypes on three sampling dates (Table I). Population density of *B. tabaci* was statistically similar and higher on chilies planted on 3rd and 11th of March, as compared to that of on 18th of March during 2008 across all the sampling dates. Higher numbers of *B. tabaci* were observed on 16th May 2008 on chilies transplanted on 3rd and 11th of March during 2008. Numbers of whiteflies adults were less than two per plant (Table II). Negligible numbers of *B. tabaci* after 23rd of the May were noted, therefore population was not recorded after this date. This might be due to rise in temperature as June is hotter than May in Multan.

Table I.- Population of whitefly, *Bemisia tabaci* (Genn.), adults per plant recorded on different sampling dates during 2007 at Multan.

Genotypes	Number of whiteflies		
	15th May	22nd May	29th May
CV-7	1.78 NS	1.61 NS	1.79 NS
Nepal-S	1.66	1.57	1.78
NARC-4	1.69	1.60	1.81
CV-4	1.79	1.56	1.83
Nepal-L	1.76	1.58	1.82

NS= non-significant

Table II.- Whitefly, *Bemisia tabaci* (Genn.), adults population on Tattapuri variety of chilies planted on different dates and sampled during, 2008 at Multan.

Date of sowing	Number of whiteflies		
	9th May	16th May	23rd May
3rd March	1.40A	1.80A	1.48A
11thMarch	1.43A	1.75A	1.33A
18th March	0.84B	0.28B	0.21B
LSD Value	0.23	0.46	0.17

*In each column means not sharing similar letters are significantly different at P = 0.05

Whiteflies have been reported as minor insect pests of chilies from cotton growing areas of the Punjab, Pakistan (Attique *et al.*, 2003). This study recorded low numbers of whiteflies on chilies. Very low populations of whiteflies have been observed on chilies in Sri Lanka (Burleigha *et al.*, 1998), but *B. tabaci* has been reported as major insect pest in India (Sanyal *et al.*, 2008).

From this study it is concluded that whitefly attacks chilies in low numbers. Such low numbers of *B. tabaci* may not cause economic damage as we observed that apparently plants did not show any symptoms of damage due to feeding by this insect. However, further research should be conducted by comparing yield and growth of chili plants kept free from whiteflies by insecticides or by caging the plants with that of infested plants. In developing countries farmers usually apply pesticides without knowing status of pests and diseases. However, no literature reports pesticide use patterns from this area. Therefore, caution is needed for application of insecticides. This study might have limitations as experiments were conducted at only one location, and cropping schemes of the farmers may affect population of insect pests particularly whitefly as it is polyphagous pest feeding upon wide variety of plants. Moreover, there is need to investigate insecticide use pattern to determine whether pesticides are applied or not.

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Phallectomy and Urethrostomy in a Paraphimosed Race Horse

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Abstract.- An eight year old gelding with a history of inability to retract his penis was presented to the clinic. Physical examination revealed granulomatous lesions on the penis. Local and systemic treatment for two weeks did not show any improvement. Animal was found negative for habronemiasis. Histological examination of the paraphimosed penis was indicative of pyogranuloma. As remedial measure amputation of the penis and penile urethrostomy were carried out. Animal recovered uneventfully after about a week and was sent back to the track.

Key Words: Habronemiasis, pyogranuloma, penis, urethra, penile amputation.

Paraphimosis, traumatic lesions, balanitis, phimosis, and paralysis are the common affections of the penis (O'Conner, 2005). Paraphimosis is characterized by inability of the animal to retract his penis into prepuce. Paraphimosis most commonly occurs in stallions as a result of breeding trauma, but geldings can also be affected. Use of phenothizine tranquilizers, systemic diseases such as equine herpesvirus-1, purpura hemorrhagica, dourine, damage to the penile innervation and severe debilitation are amongst various etiologies of paraphimosis in equids (Wheat, 1966; Neely, 1980; Walker, 1980; Blanchard *et al*., 2003).

Depending upon the condition, various treatment regimens are adopted to avert this anomaly (Brinsko *et al*., 2008). Treatment of paraphimosis includes supporting the extended penis, manually replacing the penis in the preputial cavity, use of emollients to cover and protect the fragile exposed tissues, hydrotherapy, physiotherapy of the penis, use of broad spectrum antibiotics, and surgical interventions such as preputiotomy, phallopexy, posthetomy, and phallectomy (Lisa and Soon, 2008). This short communication describes a case of paraphimosed penis with a history of trauma of back bone. Phallectomy and penile urethrostomy were carried out to correct this malady.

Materials and methods

An eight year old bay gelding with paraphimosed penis was received at the Indoor Hospital, Surgery Section, University of Veterinary and Animal Sciences, Lahore. The animal had a history of trauma of the back bone. The growth extended almost up to the $3/4^{th}$ length of penis (Fig. 1). The animal was feeding, defecating and micturating, normally. Physical examination revealed penile lesion of approximately 5 cm in length and 8 cm in width. There was no palpable swelling of parotid, mandibular and axillary lymph nodes. The faecal examination of the animal revealed no evidence of habronema infestation. Histopathological evaluation of the affected organ was indicative of numerous focal granulomas. The granulomas had characteristic club colonies, variable in size and shape, surrounded by thick zones of neutrophils (Fig. 2). The granuloma colonies and neutrophils were enclosed in capsules of fibrous connective tissue infiltrated by plasma cells, lymphocytes and macrophages (Fig. 3). Amoxicillin at the dose rate of 15 mg kg^{-1} and Prednisolone at the dose rate of 4 mg kg^{-1} were administered systemically, while tincture of iodine

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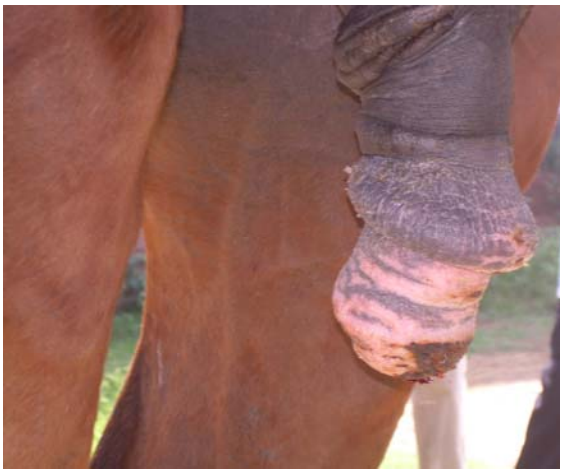

Fig. 1. Paraphimosed penis having granulamotous lesions.

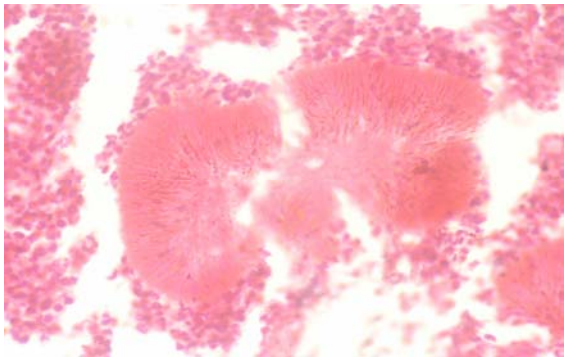

Fig.2 Histological section of penile lesions showing distinct club shaped colonies surrounded by neutrophils. (Hematoxylin and Eosin stain; X100).

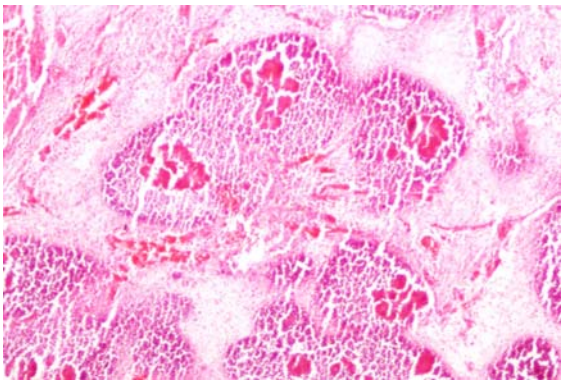

Fig. 3. Histological section of penile lesions showing numerous focal granulomas with central club colonies, enclosed in capsules of fibrous connective tissue. (Hematoxylin and Eosin stain; X40).

and Vaseline were applied topically. The treatment was continued for two weeks but the animal did not respond to this treatment. A consensus was made with the owner to amputate the affected part of penis and perform penile urethrostomy.

The animal was kept fast 24 hours before surgery. Xylazine hydrochloride 0.5 mg/kg of body weight was administered as a pre-anesthetic and Chloral hydrate 1gm/10 kg of body weight (10% solution) as a general anesthesia, intravenously. Prophylactic antitetnus serum was administered, subcutaneously. Before starting the operation, the scrotum and sheath were thoroughly cleaned with soap and water followed by application of tincture of iodine as an antiseptic. A sterile artificial insemination (AI) rod was inserted instead of urinary catheter to locate the position of urethra and two tourniquets were applied at base of the penis posterior to the level of amputation. An incision was made in the lower midline of the penis through corpus cavernosum muscle to expose the AI rod. Edges of the urethral wall were sutured to the integument of the penis, commencing on the anterior lateral aspect of the incision and continuing back to the posterior commisure. AI rod was withdrawn at this stage and penis was excised just proximal to the cranial preputial ring. Ligatures were removed two hour after the surgical exercise. Bleeding from anterior dorsal artery was noticed just after surgery which was carefully ligated. Animal was retained in the Indoor Surgery Clinic for post-operative care and monitoring for seven days post surgery. The vital signs of the patient were recorded on the day of surgery and for the following three days postoperatively (Table I). Parenteral therapy with Prednisolone 375 mg and a combination of Procaine penicillin 10000 I.U./kg, Benzyl penicillin 10,000 I.U./kg, and Streptomycin 11 mg/kg was administered intramuscularly and Phenyl butazone at dose rate of 4mg/kg, intravenously. The animal was discharged from the hospital after seven days after an uneventful recovery.

Results and discussion

Old age, illness, chronic enlargement or morbid growths either upon or within the penis itself or the sheath, the descent of calculi into the urethral

canal, paralysis of the penis, injuries, and inflammation resulting from the sexual relation are considered common etiologies of parahimosis in equids (Axe, 1905). The case under study had a history of back bone trauma which may have led to spinal nerve injury which is believed to result in paralysis and ultimately paraphimosis of the penis. Cutaneous habronemiasis (due to nematodes of *Habronema musca*, *Habronema majus* and *Draschia microstoma* species of equids) (Giangaspero *et al*., 2005; Traversa *et al*., 2007) cause granulamotous reaction which needs to be differentiated from pyogranulomas (Kahn and Line, 2005). Histopathological examination is usually carried out to confirm clinical diagnosis of habronemiasis. Histological examination of the present case, however, was not indicative of habronemiasis as no cross section of larvae of habronema was seen in histopathological section (Yarmut *et al*., 2008).

Table I.- Vital signs of the animal.

Days	Temperature (°F)	Pulse (beats / min)	Respiration (breaths / min)
1	99.0	28	14
2	98.0	36	15
3	100.0	47	25

Histopathology of penile growth was indicative of pyogranuloma. Pyogranulomatous to granulomatous inflammatory lesions of penis in horses had a wide range of etiologies. Generally it is thought that granulamatous dermatitis affect many organ systems (Sledge *et al*.**,** 2006) but an idiopathic granulomatous disease has been described to produce only cutaneous lesions in some cases (Heath *et al.*, 1990). The case in question had only cutaneous lesions on the penis. Lymph nodes were normal and there was no systemic illness observed before and after the surgery. This picture is attributed to its idiopathic origin. No attempt was made to isolate an etiological agent; however, adoption of such a procedure is likely to confirm the causative organism.

In this case an AI rod was used to locate the urethra as a modification; where as in the past urinary catheter was used for the purpose (Turner and McIlwraith, 1989).

Owing to the poor response to the medical treatment, penile amputation and urethrostomy was performed. The indications for penile amputation are neoplastic lesions, granulomas and penile paralysis (Turner and McIlwraith, 1989). Surgical treatment proved to be quite satisfactory and the horse was sent back to the track after an uneventful recovery.

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Cross Species Transfer of Microsatellite Loci in Scolytidae Species Mostly Associated with Mango (*Mangifera indica* L., Anacardiaceae) Quick Decline Disease

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Abstract.- Mango bark beetle, *Hypocryphalus mangiferae* Stebbing (Coleoptera: Curculionidae: Scolytinae) is frequently associated with mango quick decline disease in Pakistan, since it is also reported as a potential vector of quick decline disease. Hereby, we report the cross-amplification of five microsatellite markers in *Hypocryphalus mangiferae*, which were primarily characterized for other Scolytidae species. The markers were tested on 45 mango bark beetles individuals from nine different locations of Pakistan. Allelic richness ranged from two to eight alleles, the observed heterozygosity from 0.03 to 0.67, and the expected heterozygosity from 0.10 to 0.70. Two loci showed significant deviation from Hardy-Weinberg equilibrium and no linkage between the loci was detected. We also investigated cross-species amplification in *Xyleborus* spp. using the same five microsatellites . Three of the loci were monomorphic and two showed three and two alleles, respectively. Observed heterozygosity values ranged from 0.3824 to 0.0882, those for expected heterozygosity from 0. to 0.2752. These markers could potentially contribute in the management of the mango bark beetle.

Key words: Mango, bark beetle, *Hypocryphalus mangiferae*, quick decline, microsatellite loci.

Bark and ambrosia beetles (Scolytidae:Coleoptera) are among the most economically important forest insects. Approximately 6000 species of scolytids have been described in the tropical and sub tropical regions world wide (Lieutier *et al*., 2004). Typically, beetles breed in the woody plants and bore characteristic galleries either in the phloem (bark beetle) or in the wood (ambrosia beetles) (Beaver, 1967). Mostly infection by all fungi on mango is facilitated by bark beetles as a wounding agent for their penetration within the vascular system (Batista, 1960; Al Adawi *et al*., 2006) that resulted in the infection and transmission of the pathogens to the mango tree (Ploetz, 2003). It was observed that bark beetle, *Hypocryphalus mangiferae* Stebbing (Coleoptera: Scolytidae) was most frequently found in diseased (Mango Sudden Death Syndrome *i.e.*, MSDS) in early stages while *Xyleborus* sp. appeared in later stages but comparatively less in number (Masood *et al*., 2008). MSDS is regarded as one of the most serious threats not only in Pakistan but all over the mango growing regions of the world and bark beetle species are suspected to be involved as putative vectors (Ribeiro, 1980; Adwai *et al.*, 2006; Masood *et al.*, 2008). The insect vector, *Xyleborus* sp. found in *Ceratocystis* canker of deciduous fruit trees caused by *Ceratocysis fimbriata* which invades the xylem parenchyma and inner bark (phloem) as well (Webber and Gibbs, 1989). In “vascular wilts” like Dutch elm disease caused by *Ophiostoma ulmi* that was considered to be spread throughout Europe, Central Asia and North America and transported by various elm bark beetles of genus *Scolytus* (Lanier and Peacock, 1981; Brasier, 1987).

Being a potential threat for the dissemination of

MSDS, several aspects of bark beetle biology, together with monitoring and management measurements have been investigated (Masood *et al.*, 2009; Saeed *et al*., 2010). On the contrary, not so many things have been done so far regarding the study of genetic diversity of mango bark beetles that could be supportive for molecular ecology studies and ultimately lead to the formation of even more efficient management strategies.

Nuclear microsatellites can be transferred between species something that provides insight comparison among closely related species for investigating the pattern of speciation, divergence and diversity at genetic and community levels interactions (Noor and Feder, 2006; Whitham *et al.*, 2006). Therefore we tested a panel of microsatellites that were established in several scolytid species for cross amplification in *H. mangiferae* and *Xyleborus affinis* which were associated with diseased mango tree.

Materials and methods

Study site

The survey was primarily conducted in Multan District of Punjab, Pakistan. Mango orchards situated in the four nearby areas within 20 kilometers from the main city were surveyed for bark beetle during 2008-09. This survey was also extended to Southern Punjab (Sahiwal, Faisalabad), Northern Punjab (Rahim Yar Khan and Bahawalpur) and Sindh Province including the main mango growing areas: Tandojam, Tando Muhammad and Hyderabad (Table I). Each location is separated at a distance of $\geq$ 100 km from each others. We have collected at least 60 specimens of *Hypocryphalus mangiferae* and *Xyleborus affinis* from each location.

Table I.- Sampled Populations of mango bark beetles, *Hypocryphalus mangiferae* and *Xyleborus affinis*

Population of beetle	Number of individuals	GPS position	
		North (Latitude)	East (Longitude)
Hypocryphalus mangiferae			
Multan	60	30.27	71.51
Shujabad	70	29.88	71.29
Sahiwal	50	30.65	73.12
R.Y. Khan	50	28.39	70.32
Faisalabad	60	31.41	73.05
Bahawalpur	60	29.40	71.66
Tandojam	60	25.43	68.54
Tando Muhammad	50	25.46	68.72
Hyderabad	50	25.37	68.35
Xyleborus affinis			
Multan	50	30.27	71.51
Tandojam	50	25.43	68.54
Hyderabad	50	25.37	68.35
Khanewal	50	30.20	71.59

Microsatellite amplification and analysis

A total of 125 microsatellite markers primarily characterized for other Scolytidae species (Kerdelhue *et al.*, 2003; Salle *et al.*, 2003; Simon *et al.*, 2003; Gauthier and Rasplus, 2004; Stoeckle *et al.*, 2010) were examined for transfer into *H. mangiferae* and *X. affinis.* Individuals of the bark beetles (*H. mangiferae* and *X. affinis*) were collected from disease infested mango trees in different regions in Pakistan (Table I). We isolated genomic DNA from 45 beetle bodies of *H. mangiferae* and 36 inidviduals of *X. affinis* according to Hogan *et al*. (1986), following the standard phenol-chloroform extraction. PCR was performed in a total volume of 15µl with the following components: 50-60 ng of genomic DNA, 200 nM of each primer, 0.2 mM each dNTP (Solis BioDyne), 1.5 – 3.0 mM $MgCl_2$ (Table I), 1x PCR buffer (Solis BioDyne), and 0.5 U Taq DNA Polymerase (Solis BioDyne). PCR was carried out on a Mastercycler Gradient thermal cycler (Eppendorf) under the following cycling conditions: initial denaturation at 94°C for 3 min; 35 cycles of 94°C for 30 s, 50-55°C (Table II) for 30 s, 72°C for 30s, and a final extension at 72°C for 3 min. PCR products (Cy5 labeled) were separated on 6% polyacrylamide gels on an ALFexpressII DNA Analyser and scored with ALLELELINKS 1.02 software. Electrophoresis was carried out on standard plate gels with external and internal standards for exact scoring. GENEPOP4.0 software (Raymond and Rousset, 1995) was used to generate allele frequencies, to calculate expected and observed heterozygosities (H_e, H_o) and to test the loci for linkage disequilibrium and Hardy–Weinberg equilibrium.

Table II.- Characteristics of 5 microsatellite loci transfered to *Hypocryphalus mangiferae*: locus designation, primer sequences (forward primers 5´Cy5-labelled), optimal annealing temperature (T_a) and $MgCl_2$ concentration, number of observed alleles (N_A), allele size range, level of observed (H_o) and expected (H_e) heterozygositiy per locus and number (bold) of populations deviated from Hardy-Weinberg equilibrium

Locus	Primer sequences (5´→3´)	T_a (°C)	$MgCl_2$ (mM)	N_A	Allele size range (bp)	H_o	H_e	*HW*
ITY8[1]	F: CATGCATAAAACAGTTCGTT R: CGCACTAGCGCTTCTATTTA	50	3	2	317-119	0.352	0.490	
ITGT343[2]	F: ATCGTGGTGCCCGGATAAG* R: AAGCCGGCGATGTCATACAG	50	3	8	191-205	0.696	0.678	
ITY84[3]	F: GCGCTAAAGTTGGATTGTAA R: TTGCTGTGTTGTAGCAATTC	50	3	5	274-282	0.440	0.704	**1**
HHK.3.4[4]	F: GATCCCGCGACTCGAGAC R: GAGTTACAAGCGGCAGGC	50	3	2	181-187	0.028	0.441	
MS71[5]	F: TCCTTTTAAGGACCACAACT R: AGTCCGACTCTGGTAGCTTA	55	1.5	2	147-149	0.237	0.095	**1**

[1]Stoeckle and Kuehn (submitted); [2]Sallé *et al*. (2003); [3]Stoeckle *et al*. (2010); [4]Gauthier and Rasplus (2004) and [5]Simon *et al*. (2003).

Results and Discussion

Out of the 125 loci tested on 45 individuals from 9 different *H. mangiferae* populations, five loci were found to be polymorphic with two to eight alleles, averaging 3.8 alleles per locus. Details regarding their sequences, their composition and their amplification conditions are given in Table II. Observed heterozygosity values (H_o) ranged from 0.028 to 0.696, those for expected heterozygosity (H_e) from 0.095 to 0.704. The test of linkage equilibrium revealed no significant values. Tests on Hardy-Weinberg equilibrium for each locus in each population revealed one deviation in population Multan (ITY84) and one in population Tandojam (MS71) (Table II).

In addition to that we investigated cross-species amplification in *Xyleborus* spp. using the same five microsatellites as transferred to *Hypocryphalus mangiferae*. Three of the loci were monomorphic (MS71, ITY84 and ITY8). ITGT343 and HHK.3.4 showed three and two alleles, respectively. Observed heterozygosity values (H_o) ranged from 0.3824 (ITGT343) to 0.0882 (HHK.3.4), those for expected heterozygosity (H_e) from 0.5079 (ITGT343) to 0.2752 (HHK.3.4). Since only two of the microsatellites were polymorphic in *Xyleborus* spp. We did not perform the test on linkage and Hardy Weinberg equilibrium.

The molecular genetic discrimination of different populations of a bark beetle would be very helpful to support the management strategy for the species. It allows the determination of genetic relatedness of populations and therefore the identification of migration routes. Consequently the increased combat against the bark beetle in the migration routes may help to contain the further spread of the pest species.

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