Chapter V – DISCUSSION

Discussion

A. FIELD STUDIES

A1. Presence of Social Spiders in the Field Margins: Its Diet Composition and Prey Spectrum Studies

Insect pests from Paddy, Pigeon pea and Castor as well as field margins were identified and listed. The pests of paddy occurred sporadically and never attained the status of pests of major concern. In pigeon pea, podfly *Melanogromyza obtusa* was present in low numbers for the maximum period i.e. 2nd week of October till 4th week of November. In castor whitefly *Trileurodes ricini* was present throughout the season in low numbers in the field; thus the pests were found at below Economic Threshold Level (ETL) throughout the cropping season.

The spiders found in the agroecosystem (both in the cropping area as well as the field margins) were first categorized into guild structure as defined by Uetz (1977), and then segregated for different crops and field margins.

Insects belonging to eight orders were identified from collected specimens. Orders Hemiptera, Diptera and Orthoptera comprised the major portion of the insects trapped in the webs of social spider *Stegodyphus sarasinorum* Karsch. Similar composition of insects orders were reported by Pekar (2000) in a study on diet breadth of *Theridion impressum*. In the webs of orb-web spiders found along the field margins, Dipteran insects were dominant followed by Sternorrhyncha, Heteroptera and Coleoptera respectively (Ludy, 2007). The results also showed that size of insect prey trapped varied between 1-3 mm (whiteflies) to as big as 100 mm (dragonflies).

In the present study, order Hemiptera is represented by the most numerous families found in the web. The dominant family found was Aleyrodidae (whiteflies); followed by Cicadellidae (jassids), Membracidae (cow bugs), Buprestidae (Jewel beetle), and Pentatomidae (stink bugs) respectively. The high numbers of whiteflies trapped in the webs could be due to colonial nature of the insect or might be a resultant of wind which could easily transport these small sized insects with ease (personal observation). Further studies are needed to verify the exact cause of the above.

The frequency of occurrence of insects of different orders varied in different seasons in relation to the crops planted, suggesting a direct correlation of crops planted and the composition of insects trapped in the webs. In both Kharif and Rabi seasons, insects from order Hemiptera were the most abundant prey trapped in the webs; however, the family representation differed. In Kharif season family Tephritidae were abundant. In Rabi season, the dominance of Hemipteran families varied with Aleyrodidae and Cicadellidae being numerous in early part of Rabi in the months of November to January; while in the later part of Rabi in the months of February to April, Pentatomidae and Membracidae were dominant.

The increase in the diversity of prey trapped was directly correlated with the occurrence of insects in cultivated crops. In the present study a temporal change in the composition of prey taxa was found in the web. Pests like whiteflies and jassids dominated the vegetative phase of growth while Pentatomid bugs and cow bugs dominated in the reproductive phase of growth of the crop. Similiar change in the composition of prey taxa were reported by Crouch and Lubin (2000) for *Stegodyphus mimosarum*.

In addition to large sized insects, relatively smaller and medium sized insects were trapped in the webs of *S.sarasinorum*. Several large sized insects from orders Coleoptera, Hemiptera and Orthoptera were found in the webs. Studies conducted by Nentwig (1985) on social spider *Anolesimus eximius* and Rypstra and Tirey (1989) on *Anolesimus domingo* also report the presence of large sized insects in the webs of social spider.

The biocontrol potential of social spider as regulator of insects can be evaluated only after assessing the potential of the spider to trap economically important prey in large numbers (Randall, 1982).

In the present study the economic evaluation of the prey taxa trapped shows that *Trialeurodes ricini* and jassids comprise a sizable amount of the prey trapped. Adults and immature stages of *T. ricini* suck sap from the lower surface of the leaves which then wither and turn brown. Secretion of honeydew results in growth of sooty moulds. An additional concern is the transmission of Tomato yellow leaf curl *begomovirus*. Chemical and biological control methods (application of neonicotinoids or release of parasitoids, example *Encarsia formosa*) are available, but the pest is difficult to control (EPPO, 2006). Jassids feed on the leaves of the plants giving a scorched appearance to the plant in case of severe damage, which is known as 'hopper burn' (Tembhare, 1997). These insects have been known to inflict severe economic damage to cereal and vegetable crops in several parts of the world (Gerling *et al*, 2001). Another insect which is of major economic importance is *Melanogromyza obtusa*, a major pest of Pigeon pea in India (Shanower *et al.*, 1999; FAO\RLAC, 1989).

Other insects like grasshoppers, pentatomid bugs and lygaeid bugs also act as sporadic pests in several crops. This study shows that these sporadic pests can be effectively regulated by having a healthy population of social spiders along field margins. This indicates that habitat conservation along field margins can promote the colonization of natural enemies which in turn can bring down population of insect pests (Greenstone, 1999; Walter, 2003). Conservation of natural enemies can also bring down the cost of agricultural production by the reduction of agrochemical input in the fields (Oberg, 2007; Ostman *et al.*, 2003).

In India thorny shrubs and plants like *Prosopis*, *Acacia*, Cactus etc. are grown in the boundary area to keep wandering herbivorous animals like cattle and wild hogs at bay. These plants provide a suitable microhabitat for the colonization of social spiders like *S.sarasinorum*, orb-weavers like *Argiope sp.* and sheet web builders like *Cyrtophora* *sp.* The webs on the field margins are less likely to get disturbed due to human interference/ activity as compared to the cultivated area; making them an ideal habitat for conservation of biocontrol agents at low cost.

Further studies regarding the coexistence of social spiders with other species along the field margins are required so as to develop IPM protocols *using spiders* for regulation of insect pests. Manju Silwal (2000) has reported that spider species like *Phidippus* sp. (Salticidae), *Clubiona* sp. (Clubionidae), *Hippasa* sp. (Lycosidae), *Pardosa* sp. (Lycosidae), *Oxyopes* sp. (Oxyopidae), *Neoscona* sp. (Araneidae), *Argiope* sp. (Araneidae) etc. can be directly incorporated as part of biocontrol agents and strategies can be formulated for their beneficial use in the IPM program. The present study undertaken shows that social spiders form one of the potential components for the regulation of large sized insect pests in agroecosystem.

B. LABORATORY STUDIES

B1. Effect of Agrochemicals on Spiders: Direct Application

Drift levels of pesticides affect uncultivated areas of agricultural landscapes such as field margins, that are often overwintering sites for many beneficial predators (Holland *et al.,* 1999) and represent refugia in an ephemeral ecosystem (Kampichler *et al.,* 2000). More studies are necessary to assess how chemical substances commonly used in the different agroecosystems affect spiders (Marc, 1999) which are found in the agroecosystem that are invariably found at the field margins. Hence in the present study sub lethal toxicity of pesticides was investigated.

Spider webs are known to be efficient collectors of agrochemical spray (Samu *et al.,* 1992). The potential insecticidal effect on the non target arthropods within field margins is of interest because of possibilities of both over-sprays in crop fields as well as contact with spray drift in field margins (Haughton *et al.,* 2001a; 2001b).

Among the five classes of pesticides tested, spiders were found to be most tolerant to Azadirachtin (a botanical) followed by Glyphosate, Imidacloprid, Endosulfan and Methomyl. Saxena *et al.* (1984) found that NKSE (Neem Kernel Seed Extract) did not affect *Pardosa pseudoannulata,* similar to the present studies on *S.sarasinorum.* However, in residual toxicity studies at higher doses toxic influence of Azadirachtin can be seen, but the higher doses were 10 times more than the field use rate recommended for the chemical. The exposure of spiders to such high doses in field condition is quite an unlikely event.

Haughton *et al.* (2001b) studied both direct as well as indirect effect of Glyphosate on non-target spiders *Lepthyphantes tenius* under laboratory conditions as well as in field margins. They found no detrimental effect on the population by indirect exposure (field studies) and also for direct exposure (laboratory studies) suggesting that glyphosate is "harmless" to non-target arthropods.

A study on effect of Imidacloprid on spiders suggests that the predacious ability of the spiders is affected (Widiarta *et al.,* 2001). Although in the present study, predation was not studied but it was found that upon treatment with Imidacloprid (in both the routes of exposure) most of the spiders showed moulting; but they remained alive. Another study by Tanaka. *et al.* (2000) showed that susceptibility of the spiders to the insecticides differed considerably among species. Endosulfan is a chemical widely used by farmers in India. However, their toxic property towards non-target organisms is found to be selective. LC 50 values obtained for Endosulfan in the present studies are found to be near the recommended field dose which is indicative of sublethal toxicity of the chemical to non target organisms.

Methomyl is known to have a toxic profile against non-target arthropods. Methomyl is a highly toxic inhibitor of cholinesterase, an essential nervous system enzyme. It is highly toxic to bees both by direct contact and through ingestion. (DuPont, MSDS 2007)

Social spiders are vital for the regulation of insect pest having large body size. The impact of direct and residual toxicity of various classes of chemical insecticides and herbicides shows that these spiders are tolerant to the field recommended doses of chemicals. This also makes social spiders effective biological control agents which can be incorporated in IPM programs.

B1.1. Effect of Agrochemicals on Web Building Potential of the spiders

The web building activities of individual spiders were affected to varying degrees for different mode of exposure and different chemicals. It was slower and erratic for / after residual toxicity / drift spray as compared to the direct toxic effects of topical application. Tietjen and Cady (2007) observed adverse effect of sublethal doses of insecticide Malathion on the normal diet periodicity and patterns of locomotion in four species of spiders, using a propriety computer vision system equipped with artificial intelligence. Using similar equipment and methods, adverse effect of Malathion on reproductive behaviour of a Lycosid spider, *Rabidosa rabida* was also seen by Tietjen (2006). This study clearly suggested that even though there may not be direct effect or obvious effect of pesticides in form of mortality, the behaviour of the organism gets altered which shall also impact its efficacy as a biological control agent. Hence, this factor should be considered carefully while developing IPM strategies.

B2. Effect of Agrochemicals on Spiders: Study of Enzymes as Biomarkers of Toxicity Due To Agrochemicals on Spiders

The integration of pesticides in Integrated Pest Management (IPM) programs relies heavily on the effect of pesticides on beneficial organisms (Hassan, 1992 and 1994). Such information is urgently needed for the development of effective and sustainable IPM programs for a number of crops (Amano and Haseeb, 2001). Several insecticides act on specific sites within the insect's nervous system. The effect of these insecticides can be seen on Acetylcholinesterase (AChE) enzymes, which is responsible for neurotransmitter degradation at the cholinergic nerve synapse.

In the present studies, there was a significant increase in the levels of AChE found in all the Treated surviving individuals at 24 hours after exposure to sublethal doses of pesticides as compared to Untreated Control. Inhibition of AChE is a valuable biomarker assay for organophosphate and carbamate compounds (Wilczek *et al.*, 2003). In contrast, in the same study conducted by Wilczek *et al.* (2003) on spiders from heavy metal polluted areas found that the higher activity of AChE was seen in spiders collected from highly polluted as compared to weakly polluted areas. These results were similar to present study; it was supposed that animals from highly polluted areas developed better compensatory mechanisms and higher activity of AChE was possible due to increased synthesis of the enzyme molecules. Pedersen *et al.* (2002) suggested that, the AChE activity measured in spiders surviving treatment is possibly more likely to depend on the rate (sublethal dose) in which survivors recover physiologically from the toxic effect and the time taken for them to recover. This indicates that there is an active detoxification mechanism in the spiders which enabled them to survive. The increased synthesis of AChE however varied for both the exposure methods. Vial coating method of exposure showed greater response for all the chemicals compared to Topical Application method, indicating that the exposure to dried residue through the contact with appendages of the spider might be more as compared to direct application on the cuticle. This aspect needs to be explored further.

Detoxification enzymes such as Glutathione S-Transferase (GST) and Reduced Glutathione (GSH) were evaluated because many toxins inhibit or induce GST; while during metabolic transformation of xenobiotics, peroxidized derivatives and super oxide anions are formed. These derivatives and anions may cause oxidative stress if they are not eliminated by GSH (Nielsen *et al.,* 1999). Along with Glucoronidation and sulfation, GSH conjugation functions as a major detoxification pathway for many drugs and xenobiotics. Glutathione S- Transferases catalyze the conjugation of reactive electrophiles with GSH in the initial step of a detoxification pathway that ultimately results in formation of N-acetylcysteine conjugates (mercapturates) which are ultimately excreted in urine (Pearson and Wienkers 2009). Nielsen *et al.* (1997) found GST between 90 - 100ηmol $\cdot L^{-1} \cdot mg^{-1} \cdot min^{-1}$ in wolf spider *Pardosa prativaga*. In current studies, it was found to be in the similar range for social spider *Stegodyphus sarasinorum* (in Untreated Control). Higher activity of these enzymes was found in treated spiders as compared to untreated spiders. Similar results were obtained by Booth *et al.* (1998) in earthworm *Apporectodea caliginosa* when treated with Chlorpyrifos and Diazinon. GSH also contributes to reduction of toxic Lipid peroxides and transforms them into non-toxic primary alcohol through oxidoreductase reactions in presence of Glutathione Peroxidase (GPx) enzyme.

Glutathione enzymes are associated with manifestation of resistance among insects (Ramoutar *et al.,* 2009). GSH contributes to reduction of toxic Lipid peroxides and transforms them into non-toxic primary alcohol through oxidoreductase reactions in presence of GPx enzyme. Formation of lipid peroxides indicates that the integrity of biological membranes is being assaulted or has been compromised (Downs *et al.,* 2001). Enhanced activity of Glutathione enzymes with reduction in activity of LPO suggests an active detoxification mechanism in *S. sarasinorum*, at the sublethal levels of the pesticide applications. Within hours, intracellular GSH levels are known to significantly decrease in response to initial exposures to an oxidative or xenotoxic stress (Sagara *et al.,* 1998). Then, as a compensatory action, GSH levels can increase several fold compared to levels prior to the oxidative or xenobiotic stress event (Sies, 1999). Similar results were found in the present study wherein increased level of GSH is also associated with significant reduction in LPO; which also indicates an active resistance mechanism in the spiders as is often associated with Pyrethroid resistance in insects as was found by Ramoutar *et al.* (2009) in Bluegrass weevil *Listronotus maculicollis.*

Present studies on detoxification enzymes at sublethal rates for the test chemicals are indicative of an active detoxification mechanism in social spiders *S. sarasinorum.* This mechanism may be advantageous in the field condition, as it enhances the chances of survival of *S.sarasinorum* if exposed to sublethal doses of the pesticides tolerable by this spider. There is also high variability in response to different class of chemicals, with Botanicals being safe. Their response to newer chemistries like Formamidines, Oxadiazines, Diamides, Spinosyns etc. which claim safety to non-target arthropods needs to be investigated further before accrediting these species as pesticide tolerant species.