CHRONOBIOLOGICAL MANAGEMENT OF PULSE BEETLE *CALLOSOBRUCHUS MACULATUS* FAB. (COLEOPTERA: BRUCHIDAE)

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Abstract— The activity pattern of most of the insects follows circadian rhythm, with characteristic ebbs and flow. The external activity pattern is based on inner metabolic changes that alter the receptiveness of the insects. The sensitivity of insects to insecticides varies during different times of the day. In this study, the synthetic pyrethroid, deltamethrin is sprayed on *Callosobruchus maculatus* (Fab.) during early photophase (6 a.m), late photophase (12 noon); early scotophase (6 p.m) and late scotophase (12 midnight). The toxicity response varied with variations in the timing of pesticide application. *C. maculatus* adults were highly susceptible during early scotophase, when they seemed to be most active. Even at a reduced dose, a high response was observed during the early scotophase, indicating the need to spray pesticides at the appropriate time, when the pests are most vulnerable.

Index Terms— Pesticide, Chronotoxicity, Susceptibility.

I. INTRODUCTION

Insecticide residues could remain in stored grains for longer periods because storage environments are having low light or near darkness, stable or gradually changing temperature and relatively low moisture level that slow down the chemical degradation. Some residues may be as toxic as the original chemical (Hallman, 1994). Fumigants produce toxic gases that readily penetrate into the infested kernels and eliminate all insect life stages if applied properly. Fumigants do not provide residual protection, so reinfestation could occur immediately after the grain has been aired out and gas concentration falls below lethal levels (Hallman, 1994). The commonly used insecticides applied in grain storage facilities belong to organophosphate and pyrethroid chemical classes (Kernel *et al.,* 1997). Pyrethroids are currently the major insecticides used in stored grain pest control (Usmani & Knowles 2001). Pyrethroids are effective at low quantities and they are inexpensive (Nowak *et al.,* 2001). A few of photostable pyrethroid compounds such as allethrin, deltamethrin and resmethrin exhibit excellent insecticidal activity and are used in the storage systems (Ahmad *et al.,* 2003). Different pathophysiological factors that influence insecticide toxicity have been identified through toxicological studies and the quantity of insecticides to be applied is standardized (Khaire *et al.,* 1992; Ahmed *et al.,* 2001). But the chronotoxicological implications of insecticides are not properly considered and the time of administration of the toxicant is not controlled systematically. Beck (1963) showed that the time of treatment influences the susceptibility of insects to different toxins.

Callosobruchus maculatus Fabricius is one of the most important and destructive pest of almost all kinds of pulses both in the field and storage. The damage in storage is more crucial than in the field (Halberg *et al.,* 1974). Cole & Adkisson (1964) showed that the mortality produced by the insecticide is intimately related to the time of day or night at which the toxicant is applied. As a universal rule suggested by Shipp & Otton (1976) that the time of greatest susceptibility occurs at about the time of onset of increased activity. The daily changes in sensitivity to short-term insecticide treatment are dependent not only on properties of insecticide, but also on endogenous rhythmic changes in pest physiology. Halberg et al. (1974) suggested that the time of greatest sensitivity depends on the species, mode of treatment, and the insecticide used rather than being attributed to a specific moment in the life of the insects on that day. Circadian organization of detoxification mechanisms also should be carefully studied before ultimate insecticide toxicity parameters are established and released as a basis for further use. When pesticides are sprayed, spraying is done at any convenient time without paying attention to the susceptibility of the pests targeted. But chronotoxicological studies have proved that the susceptibility of pests to pesticides is a time-dependent phenomenon. Knocking down insects at the most susceptible moment would require lesser amounts of pesticides.

The objective of the present study is to find out how quantity of insecticides applied could be reduced by timing the application of pesticide. The basic preliminary studies are undertaken in *C. maculatus* and data generated are may be extrapolated to other pests also.

II. MATERIALS AND METHODS

Procurement and Maintenance of *C. maculatus*

A culture of cowpea beetle *C. maculatus* was established on the seeds of unshelled brown cowpea, *V. ungulata* in wide-mouthed plastic jars, under laboratory conditions, temperature 30 ± 2°C, RH 70 - 75%, photoperiod 11 – 13h based on the rearing method suggested by Raja & William (2008). To obtain   adults   from   the   same   stock, *C. maculatus* males and virgin female beetles were isolated and placed in separate 90mm petridishes (without seeds) and allowed a premating period of 24h. After 24h, the males and females were allowed into the same petridish and allowed to mate. Neonate adults of similar age (between 24 and 48h) were used for all the experiments. After mating sufficient time was allowed to ensure laying of at least one egg on each bean. The beans were then examined. Egg carrying beans were kept on separate eppendorf tubes for adult emergence. Emerging beetles were not allowed to mate. Newly emerged adults were sexed using the elytra pattern (Southgate et al., 1957).

Toxicity Bioassay

Freshly eclosed *C. maculatus* adults were used in the toxicity bioassay, conducted at   four different timings. The adults used in the toxicity bioassay were obtained from a stock acclimatized for about 24h after eclosion. The beetles used in the toxicity assay were isolated from the stock about 1h before the exposure. The beetles were housed in clean glass petridishs and allowed to settle down. The beetles were sprayed with different concentrations of the toxicant using a hand-held sprayer. A separate sprayers were used for each concentration. The beetles were flooded with the toxicant for exactly 20 seconds, removed using a brush, dried on a piece of paper tissue and allowed into another petridish. The beetles were checked for mortality after 24, 48, 72 and 96h of exposure. In this   study 2.8 EC deltamethrin was used, containing 2.8%w/w deltramethrin active ingredient and manufactured by Bayer Crop Science Limited, Mumbai.

Pesticide Application

The pesticide was applied in 4 different sets of concentrations, each set earmarked respectively for early photophase, late photophase, early scotophase and late scotophase. The concentrations were fixed based on pilot studies. For spraying in early photophase, the   pesticide concentrations selected were 120, 140, 160, 180, 200, 220, 240, 260 and 280μg/dl. For spraying in late photophase, the pesticide concentrations were 100, 120, 140, 160, 180, 200, 220, 240, 260, 280 and 300μg/dl respectively. For spraying in early scotophase, the pesticide concentrations selected were 60, 80, 100, 120, 140, 160, 180, 200, 220 and 240μg/dl respectively. For spraying in late scotophase, the pesticide concentrations selected were 90, 110, 130, 150, 170, 190, 210, 230, 250 and 270μg/dl respectively. Mortalities were recorded after 24, 48,

72 and 96 hours of exposure. The mortality rates were analyzed upto 96h using probit regression analysis (Finney, 1971). LC50 and LC90 values were compared.

III. RESULTS

The 24h LC_{50} of deltamethrin to *C. maculatus* was 240.61μg/dl during
early photophase; 246.22 μg/dl in late photophase, 217.91 μg/dl
early scotophase and 222.49 μg/dl in late scotophase. The 96h LC_{50} of deltamethrin to *C. maculatus* was 179.02 μg/dl during early photophase; 181.20 μg/dl in late photophase, 145.40 μg/dl early scotophase and 154.31 μg/dl in late scotophase.

Table 1
Circadian pattern of deltamethrin toxicity of
C. maculatus

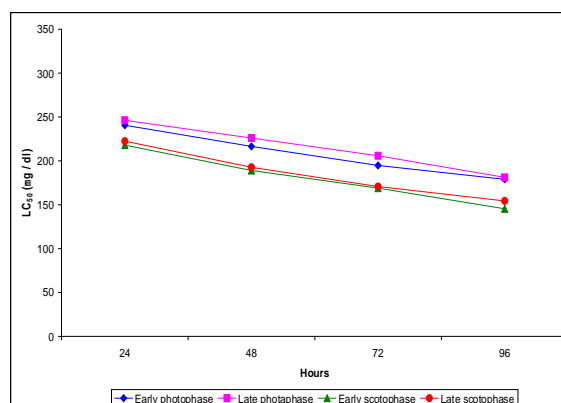| Hours of exposure | Time of application | | | | | | | |
	Early photophase (6a.m)		Late photophase (12 noon)		Early scotophase (6 p.m)		Late scotophase (12 mid night)	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
	Deltamethrin concentration (μg/dl)							
24	240.61	276.52	246.22	294.17	217.91	260	222.49	269.11
48	216.49	253.04	226.07	274.12	188.99	232.95	192.69	222.38
72	194.78	232.73	205.82	251.11	168.92	207.67	170.73	221.83
96	179.02	205.8	181.2	228.24	145.4	191.71	154.31	195.3

Figure 1: Toxicity curve patterns in C. maculatus exposed to deltamethrin

IV. DISCUSSION

When *C. maculatus* were sprayed with deltamethrin at early photophase (6am), late photophase (12 noon), early scotophase (6pm) and
late scotophase (12midnight), the mortality rates were different based on the time of spraying. In early scotophase, *C. maculatus* were most susceptible showing enhanced response to the pesticide. The normal physiological activities of diurnal and nocturnal insects are precisely timed. Cockroaches show high feeding activity during the later half of early scotophase (Lipton & Sutherland, 1970). Von Ebner's glands of the rat are minor salivary glands in the posterior portion of the tongue. They secrete two digestive enzymes, lingual lipase and amylase. In this

investigation, circadian rhythm in feeding was established under a normal 12h light/12h dark cycle, with the rats eating primarily during the dark period. At lights on, the size of the acinar cells and the areas of the inclusive secretary granules, and the amount of digestive enzyme activity (lingual lipase and amylase) remaining in the gland was significantly less than in the mid-afternoon, after very little daylight food consumption. However, after 7days of continuous light the circadian rhythm was altered the food consumption during the normal

night-time hours (5pm to 8am) went from 88percent of total 24h food consumption to 45percent and during normal day light hour (8am to 5pm) from 12percent to 55percent. These changes were correlated with histometric findings of a near reversal of the areas of acinar cells and secretory granules of photo and scotophases. Samples under contiuous light, Lingual lipase activity in the glands went from 35percent under 12h light to 6percent under continous light in the photophase and from 65percent to 39percent in the scotophase. Amylase activity also showed nearly a

reversal in activity remaining in the gland, from 36percent at 12h light to58percent at 24h light in the photophase and 64percent to 41percent for the scotophase samples. This indicate the Von Ebner's glands of the rat have a circadian rhythm of secretion and storage of secretary proteins that is subject to light entrainment similar to that seen in other exocrine glands such as the parotid and pancreas (Field et al., 1999). Diurnal changes in the neurotransmitter receptors are important for studying the receptor function in neurophysiology. The purpose of this study is to gain an insight into the regulatory mechanisms of the diurnal variation of amylase secretion. Rat salivary amylase levels showed a diurnal variation with two peaks, a marked peak at 13h and a lesser peak at 11h. This increase in salivary amylase level was completely inhibited by pretreatment of rats with the beta-adrenergic antagonist propranolol, but not by the

alpha-adrenergic antagonist phentolamine. Amylase level in parotid tissue homogenate also showed a diurnal change, but there was only one peak, at 13h. The number of maximal binding sites (Bmax) for 3H dihydroprenolol (DHA) in parotid membranes showed a diurnal variation with two marked peaks at 13 and 21h, but the affinity of parotid beta nuclear non-histone proteins in the rat parotid gland showed diurnal variation with two marked peaks at13 and 21h. These results indicate that a diurnal variation in the number of rat parotid

beta-adrenoceptors, which is presumably regulated by gene expression, is coupled with a change in salivary amylase secretion (Ishikawa et al., 1992). Rats treated with atropine and a CCK_A receptor blocker have shown that the circadian secretory pattern persists, with a rise of enzyme output during the dark period and a fall during the light period. Thus, at least in

rats, the circadian rhythm of pancreatic secretion may be regulated by very basal mechanisms, which are not dependent on CCK or the vagal cholinergic system (Keller et al., 2001).

Mosquitos actively feeds during early scotophase and some species have specific activity during early photophase (Camara, 2010). The physiological response of organisms show mild but, significant variations within the norm of reaction during different hours of a day. *C. maculatus* also shows this type of response. *C. maculatus* is least susceptible at late photophase (12 noon) (96h LC50181.19μg/dl) and highly susceptible at early scotophase (6 pm) (145.30μg/dl). The LC50 decreased by 19.75 percent. It is possible that the beetle is extremely active at late scotophase (12 mid night) secreting detoxifying enzymes against the toxicant, thus remaining highly resistant. But at early scotophase the metabolism of the insect is at a low ebb and in the absence of sufficient quantities of detoxifying enzymes like Glutathion S- transferases and other resistance mechanisms, the beetle is highly susceptible, plays an essential role for detoxification of insecticides (Leszczynski et al., 1994; Papadopoulos et al., 1999; Rufingier et al., 1999). Esterases that hydrolyze carboxylester, amide and thioester bonds in a variety of compounds. It is well established that different biological systems, including various metabolic pathways, show rhythmic changes in activity which influence either the pharmacodynamics of a chemical or the sensitivity of an organism to that chemical (Hastings & Schweiger, 1975). Application of insecticide on a calm day and during

early morning or late evening is preferable to avoid drifting of insecticide by wind and evaporation by heat (Haskell, 1985). However, the circadian peaks of sensitivity may not always be correlated with activity and metabolism (Eesa & Cutkomp, 1995). House flies and madeira cockroaches, *Leucophaea maderae* Fabricius, reared under alternating cycles of light and darkness, exhibited varying daily sensitivity rhythm to pyrethrum. Both species are most susceptible to insecticides during the last quarter of daily light span, about mid-afternoon (Sullivan et al., 1970). Adults of the yellow mealworm, *Tenebrio molitor* L. reared under 12:12 LD regime, showed bimodal susceptibility rhythm when treated with methyl parathion. Peaks of maximum sensitivity occurred at the onset of both light and dark phases (Fondacaro & Butz, 1970). Male and female pink bollworm *Pectinophora gossypiella* Saunders showed rhythmic susceptibility to azinphosmethyl. The peak of maximum sensitivity occurred one hour after the lights were on (Ware & Mc Comb, 1970). The larvae of mosquito, *Aedes aegypti* Linnaeus, are

most susceptible to chlorpyriphos, one hour after lights –off time (Roberts et al., 1974). *Drosophila melanogaster* reared under LD 12:12, showed maximum sensitivity to parathion at the end of the

scotophase and the beginning of photophase (Melou, 1985). Daily rhythmic patterns of insect sensitivity to insecticides were first reported in the German cockroach, *Blattella germanica* Linnaeus maintained under a 12L:12D photoperiod (Beck, 1963). The house cricket, *Gryllus domesticus* L. showed a daily rhythm of narcotic sensitivity when treated with ether, chloroform and carbon tetrachloride (Nowosielski et al., 1964). The sensitivity rhythm of house flies was tested using malathion, DDT and dieldrin (Frudden & Wellso,1968). The insects were most susceptible to the three insecticides at the end of the scotophase, one hour before light-on. Furthermore, the endogenous nature of the rhythm of susceptibility to DDT was tested by rearing the insects in complete darkness from the pupal stage till the time of insecticide appplication. Under such conditions the insects were found to display the same pattern of susceptibility as that recorded under the LD regime. Circadian pattern of sensitivity of insects to insecticides is absent in some insects. Houseflies maintained under different photoperiodic regimes are said to lack daily sensitivity rhythm towards malathion (Frudden & Wellso, 1968). Adults of three species of mosquito, *Anopheles culicifacies, A. annularis* and *Aedes aegypti* Lin did not display a rhythmic pattern in susceptibility to both DDT and malathion (Deobhankar & Vittal, 1984). A distinctive rhythm exists in insects which can be revealed by the analysis of the active pattern. Insects respond to ecological rhythms by adjusting their life processes into biological rhythms. Such adjustments in process rhythms can be evolutionarily advantageous when they synchronize the biology of the organism to the rhythmicity that occurs in the environment. Detailed chronotoxicological studies reveal the susceptibility of insect pests to chemical insecticides and spraying should be done at the time at which the insect will succumb to the pesticide. This reduces the amount of pesticide to be used and enhances the rate of mortality of the insect pest.

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