ALKANNA ORIENTALIS- HERBIVORE INTERACTION IN SOUTH SINAI ECOSYSTEM

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INTRODUCTION

Plants and herbivorous insects interactions have provided numerous insights into a range of important ecological and evolutionary processes (Strong *et al.* 1984; Lewinsohn *et al.* 2005; Schoonhoven *et al.* 2005; Singer and Stireman, 2005). These have included aspects of community ecology such as the role of multi-trophic interactions, including indirect and apparent competition (Holt and Lawton 1994; Denno *et al.* 1995; Fagundes *et al.* 2005; Ohgushi 2005; van Veen *et al.* 2006). Current challenges include integrating these different processes and working out the importance of direct and indirect ecological and evolutionary effects, particularly in the field (*e.g.* Ode, 2006). Many plants demonstrate an array of indirect and direct defense strategies that help to protect them from their herbivores and pathogens. Indirect defenses include the release of odours that attract the natural enemies of herbivores, whereas direct defenses may include the production of secondary compounds that impair herbivore development or repel herbivore attack (Sznajder & Harvey 2003). However, many herbivorous insects have developed counter-adaptations that enable them to feed on chemically defended plants without negative effects (Schoonhoven *et al.* 2005). In the St. Katherine area, *Alkanna orientalis* L. (Boiss.) grows in many wadis in scattered patches of different sizes separated by rocky boulders. It starts flowering early in the spring season in March before any other plants, and is the only plant in bloom in that time. *Heliothis armigera* (Hebner) (Lepidóptera: Noctuidae), the American bollworm, is an immigrant species to Britain, mainly around the southern coasts, and occurring most often in the autumn months. It is also found as a larva from time to time on tomato plants, geraniums and other plants brought in from the Mediterranean region, where it can be a pest. *Heliothis armigera* is a well known pest in some countries inspite, it is not well studied. In the study area, *Heliothis* larvae are found mainly associated with *Alkanna orientalis.* This plant/ herbivore system is a convenient one to study the insect-plant interaction. In the present study investigates the *Alkanna* / herbivore interactions throughout the effect of the plant toxin on the insect and the insect impact on the reproductive success of the plant. Such a study will be essential in elucidating any co-evolutionary pattern between the elements of this system within the ecosystem.

MATERIAL AND METHODS

Study Site: The study was carried out during 2005- 2006, in the wadis around the St. Katherine Field Station of Suez Canal University, situated in the Southern Sinai desert of Egypt, 1650 m a. s. l. (28 o 30 ' & 28 o 39 ' E, 33 o 49 ' & 34 o 03 ' N). The study took place in Wadi El Arbaein, Wadi Telah, and partialy in Wadi Tofaha systems, where vegetation was extremely sparse. The studied plant, *Alkanna orientalis* (L.) Boiss. (Boraginaceae), was the only plant in flowering in any abundance during this period. *Alkanna orientalis* (L.) is a perennial plant that is widely distributed in the eastern Mediterranean and Middle East. In southern Sinai, it is found relatively common at medium elevations, growing on rocky ground in wadis (dry river valleys), and it is locally abundant at higher elevations. Flowering occurs from late February to May with flowers borne on scorpioid cymes and remaining on the plants for several days. The plants were distributed among the rocks of the valley floor and up the lower slopes at the sides; each plant formed a discrete clump up to 1 m in diameter and bore up to 700 bright yellow flowers on any one day, though the majority of clumps were substantially smaller with around 10-60 flowers.

Ecology: Plant patches were chosen randomly; number of branches, heads, and number of infested and non-infested heads were counted for each plant. Number of seeds of infested and non-infested branches was counted. The data of two successive years were tabulated and analyzed using SPSS package. Larvae were collected and reared on *Alkanna* heads in the laboratory. The emergent adults were killed and mounted for identification. The identification of the specimens were held in the insect collection at Zoology Dept., faculty of Science, Suez Canal University

Chemistry: Extraction, isolation, and identification of the plant and insect chemicals:

Infested and non-infested plants were collected and preserved in ethanol until usage. Aerial parts (2kg) were extracted with ethanol and the ethanolic extract evaporated to dryness. The extract was chromatographed on a C_{18} column using $MeOH/H_2O$ mixtures (ElSohly, *et al.* 1997).

Insect (*Heliothis armigera*) extraction: Larvae of *Heliothis armigera* were collected and preserved in ethanol until usage. Ethanol extract evaporated to dryness. The extract was chromatographed on a C_{18} column using $MeOH/H_2O$ mixtures.

RESULTS AND DISCUSSION

The infestation of *Alkanna* with *Heliothis armigera* larvae starts early in the flowering period of the plant during February and increased gradually till May when the high level of infestation occurred (Fig. 1). This pattern was the same in both wadi systems under the study, Arbaein and Telah. Data analysis showed that the infestation increases by the increase of number of heads on the plant, and there was a positive correlation between number of heads and number of infestations per plant ($r=0.59$), (Fig. 2). Meanwhile, Fig. 3 shows that the number of infested heads associated positively with plant size ($r = 0.608$). On the other hand, fig (4) illustrates that there is a reverse effect of the altitudinal gradients on the infestation; the number of infested heads per plant decreases with the higher altitude ($r = - 0.475$); the higher the altitude, the lower the infestation. Fig.(5) shows that the larvae prefer the central site of the plant to infest rather than the peripheral part of the plant ($F_{1.289} = 23.92$, $P < 0.001$). Comparing data of three wadis, Telah, Arbaein and Tofaha, it was clear that there was a significant difference among wadis in the infestation. The infestation was higher in wadi Telah than in both other wadies. Fig (6) shows that the infestation rate was higher during first year of observation and decreased during the second year of observation. It was shown, as in fig. (7) that the infestation by the larvae varies among different wadi systems. The herbivore attack affects the plant productivity; the number of seeds was higher in the non-infested branches and decreased at the infested heads (Fig.8). Chemical analysis of both plant and insect (*Heliothis armigera*) extract revealed that the plant *Alkanna orientalis* contains flavenoides, as a plant defense. Chromatography of the insect larvae extract revealed that the larvae contain the same component (flavonoids) (Fig. 9).

The semiarid ecosystem of St. Katherine makes it a unique system, with special characteristics. It is characterized by its species richness and low abundance of individuals within the species (Semida *et al.* 2001). These conditions result in a scarcity of resources within the ecosystem; which in turn makes the small sized species subjected to a high risk of predation through their natural enemies. A counter adaptation of these species in different form (structural, behavioural or chemical)

Fig (1): Temporal pattern of herbivore infestation to Alkanna orientalis in the study area

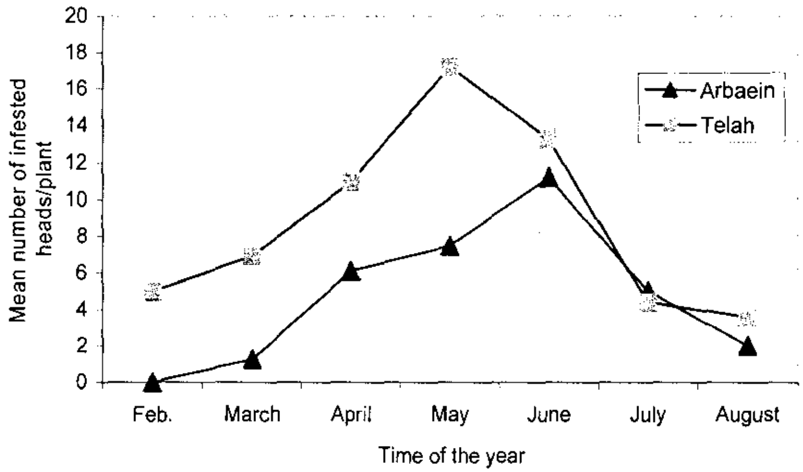


Fig (2): The relationship between number of heads on the plant and the infested heads(r = 0.596)

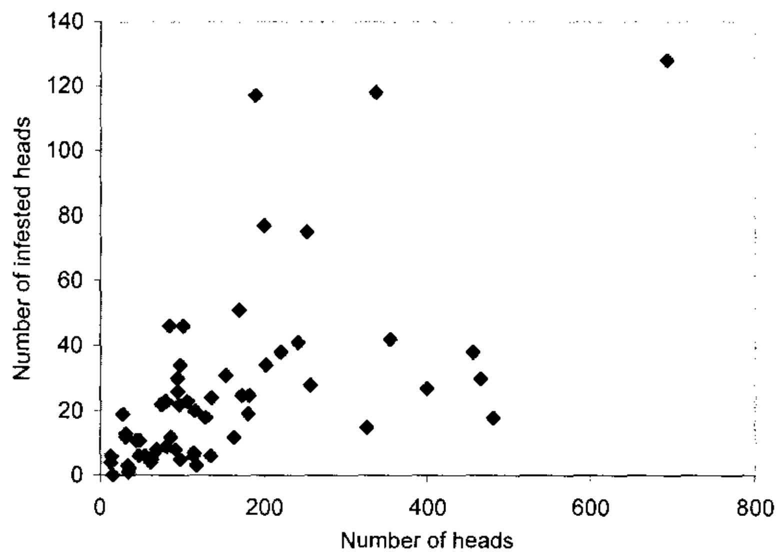


Fig (3):The relationship between the plant size and infestation (r = 0.608)

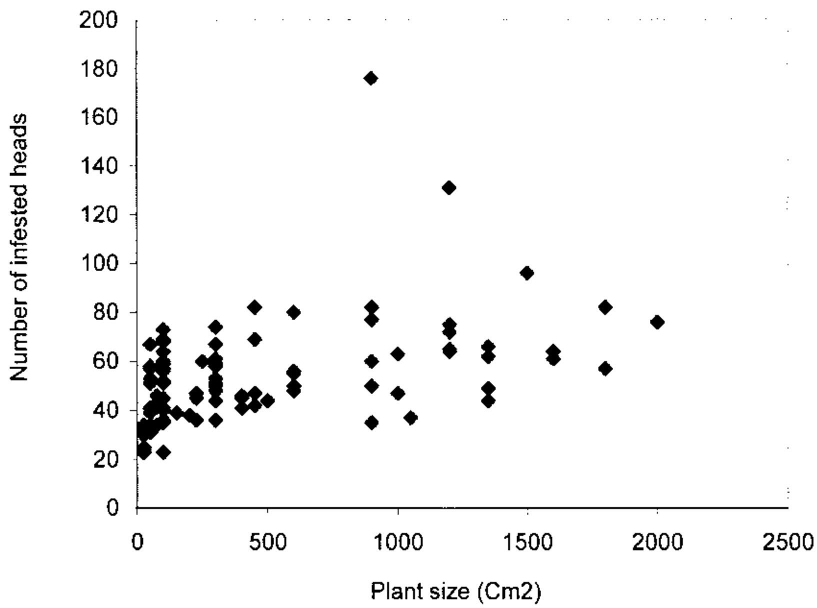


Fig (4): The relationship between altitudinal gradient and infestation (r = - 0.475)

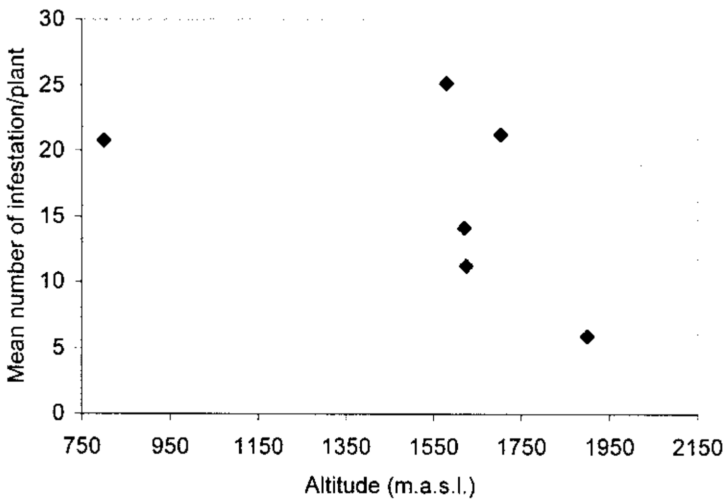


Fig (5): The plant site preference for infestation by the moth in the study area(F1,289 = 23.92, P < 0.0001)

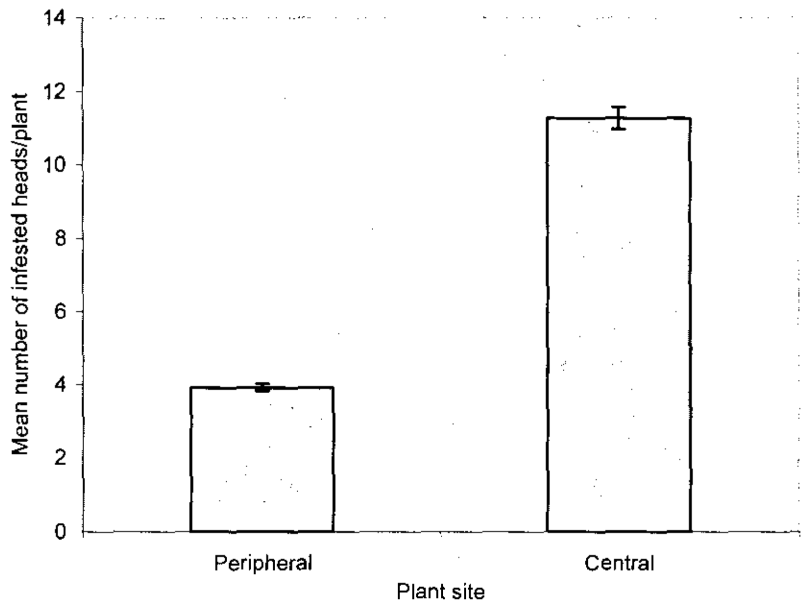


Fig (6): Temporal variation in infestation at the study area dauring the study period (F1,5 = 19.64, P<0.01)

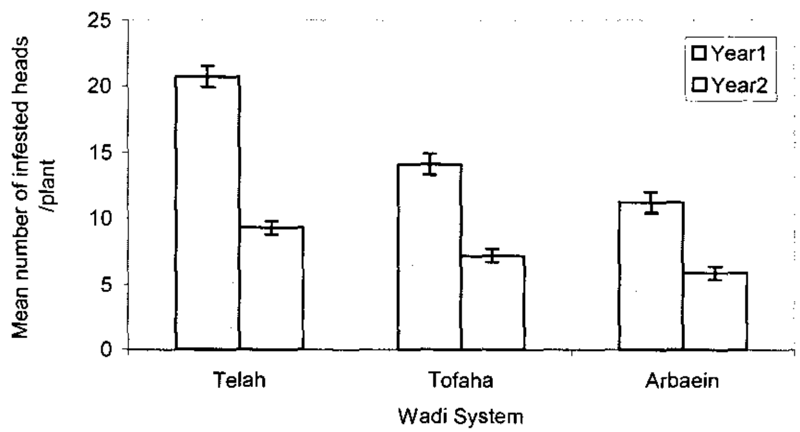


Fig (7): Spatial variation of infestation at the study area

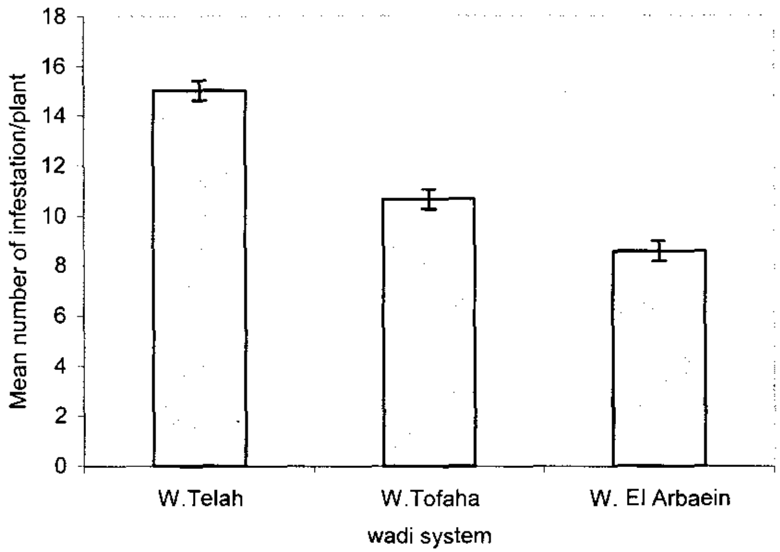


Fig (8): The effect of infestation on the reproductive success of the plant at the study area (X2=1676.83, d.f. =1,P<0.0001)

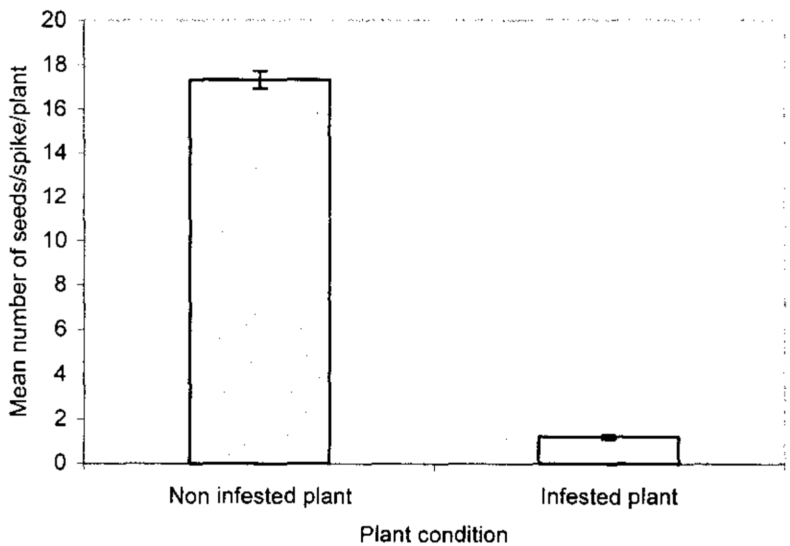
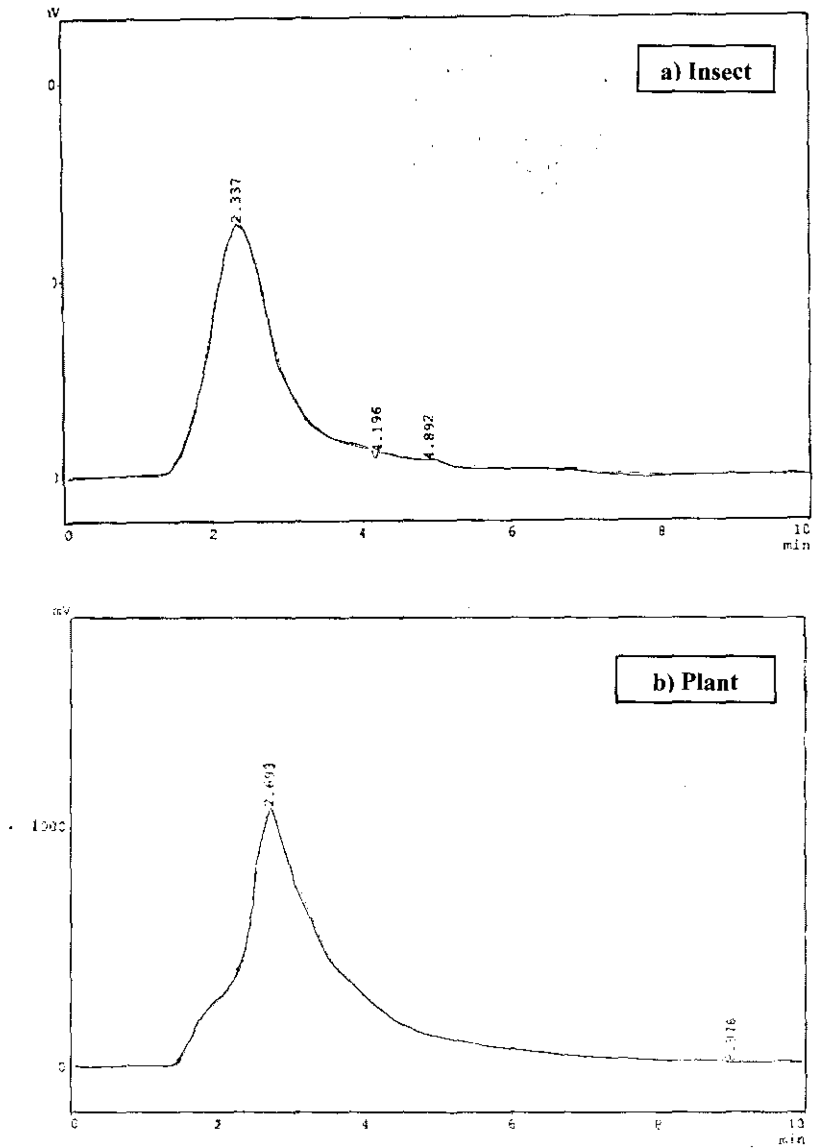


Fig. (9):HPLC trace of a) insect *(Heliothis armigera)* extract, b) plant (*Alkanna orientalis*) extract



may be adopted to avoid the risk of predation. *Alkanna orientalis* is one of the plants, which contain chemical substances as toxins; so, ungulates and many of insect species avoid feeding on the plant materials, except nectar and pollen. Meanwhile, some others adapt themselves to deal with these chemical substances without any negative impact. Moreover, they may use these substances as a defense method against other predator.

The current study shows that the *Alkanna* plant is infested by insect larvae of *Heliothis armigera* (Lepidopetra: Noctuidae) in the study area. The infestation starts early in March and it increases gradually during the flowering season of the plant; with higher infestation during May. This result suggested that the seasonal abundance of the insect (*Heliothis armigera*) is higher during May. This is strongly supported by the fact that the higher infestation in both two years was during May. The plant traits may affect the attack by phytophagous insects (Prado and Vieira, 1991). The plant stress hypothesis states that, slow growing plants or plant modules are more prone to herbivore attack because they are nutritive and less protected by defensive chemicals (White, 1984). On the other hand, the plant vigor hypothesis (Price, 1991) proposes that more vigorous, fast-growing plants or plant modules will be preferred by many kinds of herbivores that depend on high meristematic activity, modules of large size, or some chemical or nutritional property associated with vigor. The positive correlation between infestation and the number of plant heads means that the insect infestation is more likely to be found on large plants. This finding suggested that the infestation increases by the plant size which logically due to food resources availability. The negative correlation between the number of infested heads and the altitude suggested that the altitude has a reverse effect on the infestation. This effect may be due to the water resources availability at lower altitude than the higher altitude. The higher infestation at Wadi Telah rather than both other wadis may be explained by the more isolation of wadi Telah than both wadis. It is clear that the number of seeds at the infested heads was lower than seeds of non-infested heads. This indicates the negative impact of the herbivore moth on the reproductive success of the plant. The larvae feed on the plant heads which stop the plant flowering and hence the seed production. Toxic proteins and secondary metabolites are common place in different plant families, suggesting that their abundance may be due in some part to the selective pressure of herbivory (Ehrlich and Raven, 1964; Berenbaum, 1983). Various phytochemicals such as phenolics, terpenoids and alkaloids (Hartmann, 1991), proteins, protease inhibitors, oxidative enzymes and even physical barriers such as trichomes and hairs (Agrawal, 2004) can all be defense responses that are induced in response to herbivore. Several flavinoids were isolated from the plant *Alkanna orientalis*. These flavinoides thought to be synthesized by the plant as secondary metabolites to deter herbivores. The characteristics of St Katherine ecosystem, as a semi arid area, probably imply high activity of predators. This makes the insect at continues risk of predation, and hence it is necessary for the insect (*Heliothis armigera*) to be well defended. They defend themselves by sequestering and storing plant chemicals, this is inagreement with our results which indicates that the chemical analysis of the insect larvae contains plant toxins (flavonoids).

SUMMARY

The plant *Alkanna orientalis* and its herbivore *Heliothis armigera* (Hebner) were chosen to conduct the current study in the semiarid ecosystem of South Sinai. Infestation of the plant heads by insect larvae were studied and related to the plant morphology and biotic factors; such as plant size, plant site or abiotic factors such as altitude. The effect of infestation on the plant productivity was also studied. The chemical analysis of both the plants and the insect larvae fed on were also investigated. There was a positive association between the plant size and the infestation. A negative correlation was observed between infestation and the altitude. The infestation of the plant by the insect larvae has a negative impact on the plant productivity reflected as a decrease in the plant seeds.

Key words: Herbivores, *Alkanna orientalis, Heliothis armigera*, South Sinai.

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