Parasitoid *Dinarmus acutus* (Hymenoptera: Pteromalidae) Parasitizing on bruchid beetle, *Acanthoscelides macrophthalmus* on Leucaena Tree at Alexandria, Egypt

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ABSTRACT

Acanthoscelides macrophthalmus (Schaeffer) (Coleoptera: Chrysomelidae: Bruchinae) hold over high host specificity to *Leucaena leucocephala* (Lamark) deWit (Fabales: Fabaceae) at Alexandria (Egypt). The infestation rate of the seed beetle and its population fluctuation were determined under natural conditions. The rates were low between February and May 2017, While, it increased and reached a peak in August and decreased in the next January. The parasitoid *Dinarmusacutus* (Thompson) (Hymenoptera: Pteromalidae) emerged in August with high numbers and reached a peak in September, 2017.

The current study recorded *A.macrophthalmus* as a new host of *D. acutus* at Alexandria. The average survival rate of *A. macrophthalmus* and *D. acutus* until the death of adults were 30±3 days and 21±3 days, respectively.

This study presented some baseline information regarding the development of *A. macrophthalmus* that may be beneficial as a biological control agent of *L. leucocephala* at Alexandria, Egypt. In contrast, if *L. leucocephala* was considered as beneficial tree, the mass rearing of *D. acutus* should be done to control *A. macrophthalmus.*

Keywords: biological control, population fluctuation, *Leucaena leucocephala*, seed bruchid beetle, *Acanthoscelides macrophthalmus*, parasitoid, *Dinarmus acutus*.

INTRODUCTION

Acanthoscelides macrophthalmus (Schaeffer) (Coleoptera: Chrysomelidae: Bruchinae) is a seed predator for controlling the weedy shrub *Leucaena leucocephala* (Lamark) de Wit (Fabales: Fabaceae). Therefore, it is considered to be both a pre- and post-dispersal 'seed predator'(Kergoat *et al.,* 2005; Raghu *et al*., 2005; Vassiliou and Papadoulis, 2008; Tuda *et al.,* 2009) studies were carried out on this beetle in several countries such as Australia (Raghu *et al*., 2005), in South-East Asia (Wu *et al*., 2013) and in Ethiopia (Yirgu *et al*., 2015). Bruchinae beetles are highly host-specific seed feeders during the larval stage (Shobam and Olckers, 2010; Wood *et al*., 2017).

Leucaena leucocephala is a tree/shrub cultivated for fodder, green manure, reforestation, windbreak, fuel, pulp, erosion control and vegetable crop (Barrett, 1990). On the other hand, It eventually became one of the 100 worst invasive alien species in more than 20 countries where it was introduced (Lowe *et al.*, 2000 and Walton, 2003). Its seed production is heavy (up to 1700 pods/tree) and each pod containing approximately 20 seeds (Raghu *et al.,* 2005).

The species richness and evenness of parasitoids attacking the bruchid beetle, *A. macrophthalmus* feeding on *L. leucocephala* seeds were determined. A total of 1420 parasitoids (Hymenoptera) belonging to four families and five subfamilies were recorded (Wood *et al*., 2017). *Dinarmus acutus* (Pteromalidae) is a primary parasitoid of many species of bean weevils (Bruchinae) and distributed for the fauna of the world (Gupta *et al*., 1997; Andriescu and Mitroiu, 2004; Ghahari *et al*., 2010; Tselikh, 2012; Noyes, 2014; Dzhanokmen, 2015; Alrubeai, 2017; Tselikh and Kostjukov, 2017).

A. macrophthalmus was first recorded in Egypt by Mohammad (2017). But, there are no studies about the efficacy of it as a biological control agent. This study aims to determine the population fluctuation of *A. macrophthalmus* and its parasitoid, *D. acutus*on *L. leucocephala* at Alexandria, Egypt. This information is necessary to use *A. macrophthalmus* as a biological agent for control invasive weed *L. leucocephala* or a host for rearing the parasitoid *D. acutus* in a mass production for biological control.

MATERIALS AND METHODS

1-Natural infestation of *Acanthoscelides macrophthalmus* on *Leucaena leucocephala*:-

Infestation by *A. macrophthalmus* was followed in five trees of *Leucaena leucocephala* planted in different parts around the Faculty of Agriculture, University of Alexandria, ElShatby district. Weekly random pick up of 50 ripe pods on 5 unlike trees (10 pods from each tree) of *L. leucocephala* were collected throughout the year 2017. The ripe pods were transferred inside paper packages to the laboratory. In the laboratory, the collected pods and their seeds were examined carefully under a dissecting microscope at 20 to 40 magnifications. The pods and seeds of *Leucaena* infested by *A. macrophthalmus* contained exit circular neat holes, but bigger in diameter than the exit hole by parasitoid *Dinarmus acutus* (Fig. 1). According to Sanders *et al.* (2013) *A.macrophthalmus* establish circular exit holes middling 1.7 mm in diameter in both seed and pod. While, holes from the parasitoid wasp were performed similar to the bruchid beetle exit hole but smaller mean 0.6 mm in diameter.

Infested pods and percentage of natural infestation (%predation rate) of the pods and seeds by beetles of *A. macrophthalmus* were counted. From the counts, mean number of seed bruchid beetle per pod and percentage of unemerged beetles of *A. macrophthalmus* (the mummified remains as adults in exit hole) (Fig. 2) per pod were determined.

2- Population fluctuation and development of *A. macrophthalmus* and its parasitoid wasp, *Dinarmus acutus*:-

The present work was carried out under prevailing natural field conditions in six locations at Alexandria [gardens of El-montaza (31^o 17’18.582” N, 30^o57’488”E), Antoniads (31^o 13’18.004” N, 29^o56’48.042”E), Faculty of Agriculture El-Shatby (31^o12’18.62”N, 29^o55’8.86”E), Police hospital (31^o 9’55.223.582” N, 29^o56’0.005”E), Alexandria Agricultural road (30°48.0316 N, 30°59.8407 E) and Smouha (31^o 21‘29.17” N, 29^o98‘78.06” E)], Egypt. Climatic mean values during the year of 2017, obtained from data reported by the weather station: 623180 (HEAX), Ministry of Agriculture as showing in Table (1). This ecological zone is characterized by monthly mean temperatures from 15 to 32°C throughout the year and relative humidity fluctuated between 65% and 87% during this season at Alexandria, Egypt.

(A) (B)

Figure 1: (A) The exit circular holes of *Acanthoscelides macrophthalmus* on pods and seeds of *Leucaena leucocephala*.
(B) The exit hole by parasitoid, *Dinarmus acutus* on seeds.

(C)

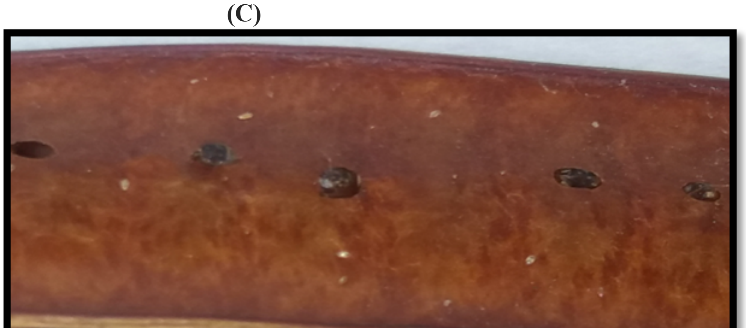

Figure 2: The mummified remains of un-emerged *Acanthoscelides macrophthalmus* adults in exit hole on pods of *Leucaena leucocephala*.

The population fluctuation of *A. macrophthalmus* and its parasitic wasp, *Dinarmus acutus* were estimated from January 2017 on *L. leucocephala*. Five of *leucaena* trees were selected in each of the chosen locations with fix-space plant established. From each tree, 10 pods were collected monthly (50 pods per location). Samples of mature pods were taken and put in labeled paper packages. Mature pods were placed in covered plastic jars in the ambient conditions (28 ± 2° C and 75 ± 5% RH) in the laboratory. Each plastic jar was fixed to dark muslin cloth and covered in the top by plastic cover provided with glass tube for trapping adults of *A. macrophthalmus* and *D. acutus* (Fig. 3). Emerging bruchid beetles and the parasitoid in the glass tubes were picked up and counted daily. The removed adults of *A. macrophthalmus* and *D. acutus* from each tube were put separately in labeled glass vial and covered with muslin cloth, then observed daily until death of adults. Male and female adults of *A. macrophthalmus* and *D. acutus* were identified by morphological traits (Fig. 4). This information is essential to calculate the sexual ratio and survival rate of *A. macrophthalmus* and *D. acutus* in nature conditions at Alexandria, Egypt.

Table 1: Climatic mean values during 2017 at Alexandria, Egypt.

Season 2017	Climatic mean values	
	Temperature (^{o}c)	RH%
January	17.3 ± 1.9	70%
February	18.5 ± 2.6	68%
March	21.1 ± 2.01	65%
April	23.6 ± 2.6	65%
May	27.8 ± 3.9	67%
June	29.5 ± 2.5	81%
July	30.8 ± 2.7	80%
August	31.9 ± 0.6	78%
September	29.2 ± 1.2	87%
October	27.7 ± 5.6	77%
November	24.1 ± 4.7	80%
December	19.2 ±1.3	70%

(A) (B)

Figure 3: (A) Plastic jar provided with glass tube for trapping adults of *Acanthoscelides macrophthalmus* and *Dinarmus acutus*.
(B) Plastic jarscovered with dark muslin cloth.

♀ ♂ ♀ ♂

(A) (B)

Figure 4: (A) Adults (♀ and ♂) of *Acanthoscelides macrophthalmus*.
(B)Adults (♀ and ♂) of *Dinarmus acutus.*

3- Morphometeric measurements of the hymenopterous parasitoid *Dinarmus acutus*:-

Measurements of ten individuals from each males and females of the parasitoid *Dinarmus acutus* were conducted using research microscope and micrometer lens. The means of ten measurements in mm were obtained with Standard deviation.

4- Statistical analyses:-

The mean numbers of the distinct parameters of the experiments were calculated using the analysis of variance test (ANOVA), with the mean separation at 5% level of significance using computer program Costat according to the method of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

1-Natural infestation of *Leucaena leucocephala* by *Acanthoscelides macrophthalmus*:-

The mature pods of *Leucaena leucocephala* infested by *A. macrophthalmus* were 72 ± 7.8% from all the collected pods and the in general average percentage of seed loss due to infestation by this beetle were 80 ± 7.7%. Emerged percent of *A. macrophthalmus* adults were 69 ± 7.3% from the pods on both sides, which contained the exit circular holes to the bruchid beetle. But, the mummified remains of un-emerged *A. macrophthalmus* adults in exit hole on pods of *L. leucocephala* were 11 ± 7.6% from both sides (Table 2). The present study showed that uninfested pods contained (7.9 ± 0.2) intact seeds, while pods infested by this beetles included significantly ($P < 0.05$) reduced number of intact seeds (2.6 ± 0.2). Previous studies indicated that between 8 and 30 seeds are created per uninfested pod (Walton, 2003; ISSG, 2006; Sankaran, 2007). An average of 14.67 seeds per pod was collected (Sanders *et al.*, 2013).

The present data shoewd that *A. macrophthalmus* reduce seed production in *L. leucocephala*. Green and Palmbald (1975) established that the seed predation by *Acanthosce iides fraterculus* (Horn) overstep 60% in *Astragalus cibarius* Sheld. And *Astragalus utahensis* (Tom) populations, and reach nearly 100% (Rogers and Garrison, 1975) in natural plant communities, and this variation related to years and locations (Baskin and Baskin, 1977). Seed predation of *Glycyrrhiza lepidota* Pursh vary between 7 and 71% with an general average of 41 ± 2% (Boe and Wynia, 1985). So, Legume seed predation by bruchid beetles may overstep 50%. While, Boe *et al.* (1989) found 36% of the pods included *Acanthosce iides perforatus* (Horn) (Coleoptera: Bruchinae) in both lobules and larvae of the seed predator taken place in 77% of the mature pods.

Table 2: The infested pods and percentage of predation rate by beetles of *Acanthoscelides macrophthalmus* on *Leucaena leucocephala* during 2017 at Alexandria, Egypt.

%Infested pods	Mean number of beetles / pod ±S.E	% Predation rate by beetles		% unemerged beetles from pods
		pods	seeds	
72 ± 7.8	13.25 ± 2.1	69 ± 7.3	80 ± 7.7	11 ± 7.6

-Means significantly different at the 0.05 level.
- Means of 5 different trees of *L. leucocephala*; 10 pods per tree (50 sample).

Johnson, 1981 stated that seeds of *L. leucocephala* are small (about 1.5 mg/seed), and mean number beetles of *A. macrophthalmus* devoured seeds about 13.25± 2.1 per pod, one or more bruchid larvae feed and develop inside a single seed in large-seeded legumes. While, the larvae of *A. perforatus* devoured from 2 to 12 seeds (Boe *et al.*, 1989).

A. macrophthalmus has been invaded West Africa, feeding on the host plant, *L. leucocephala* (Delobel and Johnson, 1998). Since 1999 this bruchinae species has been introduced in South Africa for biological control of *L. leucocephala* (ARC-PPRI, 2003; Olckers, 2004). Only, *A. macrophthalmus* was used as bio-agents control of weedy plants and emerge from the seeds of *L. leucocephala* (Tuda *et al.*, 2009). *A. macrophthalmus* emerged from *L. leucocephala* was able to place eggs and hatching larvae were able to feed on *L. leucocephala* seeds, after that bruchid beetles emerged from these seeds. Our review of the issued work indicated that *A. macrophthalmus* had high host specificity to genus *Leucaena* not in native regions only but also in introduced regions (Pfaffenberger and Johnson, 1976; Johnson, 1979; Jones, 1996; Hughes and Johnson, 1996; Delobel and Johnson 1998; Tuda *et al.*, 2009; Olckers and Welsford, 2013; English and Olckers, 2014).

2- Population fluctuation of *A. macrophthalmus* and its parasitoid, *Dinarmus acutus* during 2017 at Alexandria, Egypt:-

This study identified *Dinarmus acutus* as a parasitoid of *A. macrophthalmus* and showed its direct effect on the reproductive capacity of the plant *L. Leucocephala* and population fluctuation of *A. macrophthalmus*. These findings propose that explanation of the interactions between host plant, bruchind beetles, and the hymenopterous parasitoid is important to comprehending their ecology and population fluctuation. The effect of bruchid beetles on long-term seed production of native legumes and the efficacy of hymenopterous parasitoids for reducing seed losses can be decided after more extend evaluation.

2-1.Temporal variation of *A. macrophthalmus* and parasitic wasp, *D. acutus* density

In the laboratory, collected *L. leucocephala* pods from six locations showed that *A. macrophthalmus* is present all the year. The number of emerged *A. macrophthalmus* adults was low during the first half of the season 2017, between February and May. Number of adult's emergence increased from June and arrived a peak in August, and starting to decrease up to January. On the other hand, adults of parasitoid began to emerge in August with high numbers and reached a peak in September, but only a few adults of *A. macrophthalmus* emerged during this time of 2017 (Fig. 5).

The present results agree with that of Elder (2002) and Effow *et al.* (2010) who mentioned that the females of bruchid beetle deposit their eggs throughout the year on ripe pods. The infestation rates of this beetle were low between February and March, corresponding to the majority of *L. leucocephala* trees partially loses their foliage and dry pods. These old pods did not prefer to be selected by the females for oviposition. But, the rates of infestation increased in April and a maximum rate of assault taken place between August and December (64 and 72% of the pods were charged). This period corresponds to the good flowering and new pods that are preferentially chosen by females for oviposition

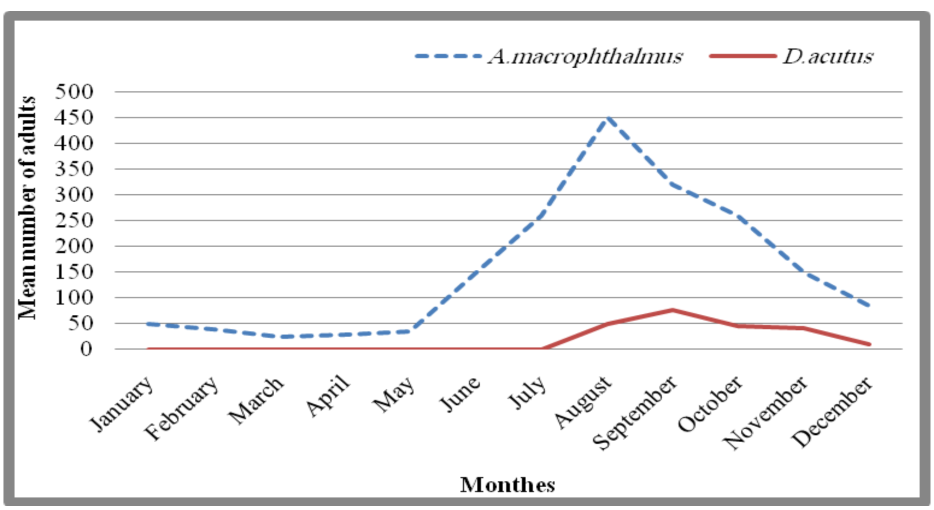

Figure 5: Population fluctuation of *Acanthoscelides macrophthalmus* and its parasitoid, *Dinarmus acutus* during 2017 at Alexandria, Egypt.

The parasitoid *Dinarmus acutus* was parasitizing *Acanthoscelides macrophthalmus* as a new host recorded throughout the present study in Alexandria, Egypt. This parasitoid has been breeded from *Bruchidius ater* (Marsham) and *Bruchus brachialis* Fahr. (Krombein *et al*., 1979) and registered from many of seed legumes including *Acanthoscelides fraterculus*, *A. griseolus* (Fall) (Johnson, 1970) and *A. perforatus* (Boe *et al*., 1989). *D. acutus* had a straight effect on *L. leucocephala* by decreasing the number of seeds devoured by larvae of *A. perforatus*. Since larvae of *A. perforatus* consumed more food of seeds 85% than those parasitized by *D. acutus* 62% (Boe *et al.*, 1989). Fabres and Reymonet (1991) found that the ectoparasite *D. Acutus* emerge in small numbers in autumn of the same year and then abundantly in spring of the following year after a diapause in the last larval stage. While, few of the *D. acutus* adults were found during July or early August, and more between mid- August to September (Leong and Dickason, 1975).

2-2. Adult survival of *A. macrophthalmus* and its parasitoid, *D. acutus* in the laboratory

In laboratory, the survival rate of *A. macrophthalmus* and its parasitoid, *D. acutus* until the death of adults were 30±3 days and 21±3 days, respectively. The adult female of both *A. macrophthalmus* and *D. acutus* lived from two to three weeks.

During the development of *A. macrophthalmus* and the parasitic wasp, *D. basalis* in laboratory, the total developmental period (egg to adult) continued 33.8 ± 2.9 days and 33.4 ± 2 days on pods and seeds, respectively. These means were different according to substrate of oviposition; which was longer on pods than on seeds. The development was low at the first stages because of very hardness of the tegument of *L. leucocephala* seeds that can create a barrier for the perception of the first instars inside the seed (Mondedji *et al*., 2002). This is the main reason to describe the long development of *A. macrophthalmus* when the eggs are laid on pods as compared to on seeds (Effowe *et al*., 2010).

During their lifetime, the sex-ratio (♂: ♀) was approximately 1: 1.5 and 1: 2.7, when calculated from the adults of *A. macrophthalmus* and its parasitoid, *D. acutus* emerged from collected pods, respectively, during this season of 2017. Under laboratory conditions, a female of *D. acutus* produced 35 offspring in a sex ratio of 1:1. Females preferred mature pods infested with bruchid and 4^{th} larval instars. The egg was deposited inside the cavity of seed on the body surface of the bruchid host. The larval stage of this external parasite consists of 3 larval instars and overwintered as a 3^{rd} instar inside seeds. The life cycle required for completion from egg to adult about 24 days and 10 months in non-overwintering and overwintering brood, respectively (Leong and Dickason, 1975). Also, Effowe *et al*. (2010) found that the sex-ratio of *A. macrophthalmus* on pods and seeds were 50.3% and 50.9% females in southern Togo (West Africa), respectively.

3- Morphometeric measurements of the hymenopterous parasitoid *Dinarmus acutus*:-

During this study, observation showed that the size of *A. macrophthalmus* adults was different between beginning and the end of the season. Haga and Rossi (2016) reported that the *L. leucocephala* seed characteristics influence on the body size and of *A. macrophthalmus*. According to that variation in measurements of body size of the parasitoid *D. acutus* adults was appeared. So, the measurements of males and females of the parasitoid *D. acutus* adults were accomplished and the mean ± Standard deviation in mm were given in Table (3).

Future studies are required to determine the relation between the size of *A. macrophthalmus* larvae and the size of its parasitoid *D. acutus*.

CONCLUSION

The bruchid beetle, *Acanthoscelides macrophthalmus* (Schaeffer) (Coleoptera: Chrysomelidae: Bruchinae) oviposit on seed pods of the tree *Leucana leucocephala* (Lam.). The beetles lay their eggs on seed pods and on loose seeds of its host. Hatching larvae burrow into the seeds to consume the contents, emerging as adults. It was released as biological control agent to reduce the invasive Neotropical tree *Leucaena leucocephala* in certain countries.

Table 3: Mean ± S.D. measurements (mm) of males and females of the parasitoid *Dinarmus acutus* adults on its host *Acanthoscelides macrophthalmus* at Alexandria, Egypt.

Mean± S.D. of the parasitoid *D. acutus* adults					
Male			Female		
L. Body	L. Ant.	L. Wi.	L. Body	L. Ant.	L. Wi.
0.2153±6.7	0.54±3.4	0.129±2.9	0.371±16.6	0.106±1.2	0.214±2.1

*Abbreviations: L. Body= The length of Body, L. Ant. = The length of Antenna, L. Wi. = The length of Wing.
*data from 10 individuals.

The data in this study introduce, some information regarding the development of *A. macrophthalmus* that may be beneficial for its promotion as a biological control agent, if *L. leucocephala* was considered as invasive weed in Egypt. The parasitoid, *Dinarmus acutus* (Thompson) (Hymenoptera: Pteromalidae) was recorded for the first time on *A.macrophthalmus* at Alexandria, Egypt. If the Neotropical tree *L. leucocephala* consider as beneficial agroforestry, *A. macrophthalmus* could be used as a host for rearing the parasitoid *D. acutus* in a mass productionto biocontrol *A. macrophthalmus*.

AKNOWLEDGMENT

Authors deeply thank Dr. Nevein Gad Allah professor of Entomology - faculty of Science - Cairo University who identify the parasitoid, *D. acutus*

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طفيل ***Dinarmus acutus*(Hymenoptera: Pteromalidae)** المتطفل على خنفساء ***Acanthoscelides macrophthalmus*** المتغذية على بـــذور الليوسينا فى الإسكندرية، مصر

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تتطفـــل حـــشره *Acanthoscelides macrophthalmus*(Schaeffer) (Coleoptera: Chrysomelidae: Bruchinae) على قرون أشجار الليوسينا *Leucaena leucocephala* (Lamark) deWit (Fabales: Fabaceae) فى مـــصر. تمـــت دراســـه معـــدلات الإصـــابة والتذبـــذبات العدديـــة للحـــشرة والطفيـــل *Dinarmus acutus* (Thompson) (Hymenoptera: Pteromalidae) تحت الظروف الطبيعية خلال عام ٢٠١٧. أظهرت النتائج أن معدلات الإصـــابة كانت منخفضة خلال شهرى فبراير حتى شهر مايو ووصلت إلى قمة التعداد فى شهر أغسطس وإنخفضت مـــرة أخرى فى شهر يناير. الطفيل بدأ فى الظهور فى شهر أغسطس بأعداد كبيرة ووصل لقمة التعـــداد فـــى ســـبتمبر ٢٠١٧.

أظهرت الدراسة الحالية أن خنفساء *A. macrophthalmus* تعتبر عائل جديد للطفيل *D. acutus* فى الإسكندرية. وقد بلغت معدلات البقاء لخنفساء *A. macrophthalmus* والطفيل *D. acutus* حتى موت الحشرات الكاملـــة ٣٠ ± ٣ يوم و٢١ ± ٣ يوم على التوالى.

أظهرت النتائج فى هذه الدراسة بعـــض المعلومـــات الأساســـية والتـــى تـــشير إلـــى أن تطـــور خنفـــساء *A. macrophthalmus* التى تعتبر كعنصر مكافحة حيوية نافع إذا كان وجود نبات الليوسينا كنـــوع مـــن الحـــشائش الدخيلة على الإسكندرية بمصر وعلى الجانب الأخر إذا اعتبرنا نبات الليوسينا كـــشجرة نافعـــة للزينـــة أو ضـــد التصحر أو كمصدر للعلف وغيرها من الفوائد فأن التربية المكثفة بأعداد كبيرة للطفيل *D. acutus* يجب أن تـــتم للقضاء على خنفساء *A. macrophthalmus* التى تسبب فقد كبير لبذور الليوسينا.