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First record of *Cylindromyia (Conopisoma) rufipes* of subfamily Phasiinae (Tachinidae : Diptera) as facultative endoparasitoid of *Nezara millierei* (Pentatomidae: Hemiptera) in Egypt

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Abstract:

The present study reported the tachinidae fly *Cylindromyia (Conopisoma) rufipes* (Meigen) of subfamily Phasiinae (Tachinidae : Diptera) as an endoparasitoid attacking colony of the *Nezara millierei* Mulsant and Rey (Pentatomidae: Hemiptera) for the first time. This case of parasitism was observed inside rearing cages of the *N. millierei* at the Faculty of Agriculture, Cairo University, Giza, Egypt in October 2020. We firstly identified adult individuals of *N. millierei* which were found moving erratically here and there within the cages using relevant identification keys. To verify that they are parasitized by the same parasitoid and no other parasitoids were detected, some of the parasitized bugs were transferred to a separate cages at the same laboratory conditions, and the developmental stages of the dipteran parasitoid were observed by the adult emerges. The present investigation reveals that *C. rufipes* could have a potentiality to control *N. millierei*, and further studies should be carried out to assess the effectiveness of this fly as a biocontrol agent.

Introduction

The stink bug *Nezara millierei* (*Acrosternum millierei*) (Meigen, 1824) (Hemiptera: Pentatomidae) is belonging to sucking insect, they attack all parts of the plants and prefers growing shoots and developing fruits. They are fed by injecting water; saliva containing digestive enzymes and suck out liquefied contents. Also, this insect causes indirect damage to plants by providing an entry slot for pathogenic and decaying organisms at the feeding punctures (Panizzi, 1997).

Gözüaçik (2018) detailed that *N. millierei* was invasion caper plants amid the dry period. Sucking show up on bud and natural products of capers amid this time. Bud deformation due the spots shaped the sucking and the color alter caused by the proteins emitted and drop therefore, diminished the natural product quality in this way diminished the financial esteem of the editors. In Egypt, Linnavuori (1964) listed that, *N. millierei* (*A. millierei*) collected from Cairo, Suez desert road and Kharga on cultivated field in 1962. Later, Linnavuori (1993) recorded that *N. millierei* distributed in Iraq and had a wide range in the Middle East and the Ethiopian region.

Rabitsch (2008) reported that a single example of this Mediterranean

species expanding to tropical Africa was captured in a light trap in 2004 in Zala County and in Hungary (Kondorosy, 2005). This status merited further consideration because it may get to be setup beneath current climatic condition in mild Europe as watched for *Nezara.*

Ghahari *et al.* (2014) reported that *N. millierei* (*A. millierei*) is widely distributed in Iran and have many plant hosts. According to Ribes and Pagola-Carte (2013) *N. millieri* lives on or under a great number of plants belonging to Pinaceae, Cupressaceae, Euphorbiaceae, Anacardiaceae, Fagaceae, Araliaceae, Ranunculaceae, Fabaceae, Poaceae, Cistaceae, Oleaceae; these authors recall that in Saudi Arabia *N. millieri* lives in deserts biotopes but that in Israel it has been collected in cool places. *N. millierei* is the vector of *Nematospora coryli* as plant pathogen to pistachio trees (Safavi, 1974). Also, an unknown species, *Acrosternum* sp., were recorded by Hashemi Rad *et al*. (2000 and 2002) from Kerman as the pest of pistachio which we suspect that it was *N. millierei* Tanchinid family is the second largest family in order Diptera and include approximately 8500 species. Their larvae are endoparasite of arthropods and some species their flies lay a role as pollinators (Tooker *et al*., 2005; Al-Dobai *et al*., 2012 and Blaschke *et al*., 2019).

Most tachinid flies are larger than a house fly and noticeably bristlier, but they range in size from 2-20 mm. and between the family there is a huge difference of form, colors and degree of bristling. All tachinid flies are parasitoids in their larval stage and their hosts all belong to the Arthropoda, almost exclusively the Insecta. Tachinids select as their hosts larval and adult Coleoptera, Orthoptera and Hemiptera, the last one order described as a main host by several species of the subfamily

Phasiinae (Dupuis, 1963). There are four subfamilies traditionally recognized in the family Tachinidae, namely, Dexiinae, Exoristinae, Phasiinae, and Tachininae (El-Hawagry, 2018). The subfamily Phasiinae are the smallest species (O'Hara and Wood, 2004). Phasiinae vary significantly in size (2 –17mm) and brightly colored fly with a red to orange abdomen often accented by various black markings (Blaschke *et al.*, 2019). They have specialized antennal receptors that are extremely sensitive to the host pheromones (Aldrich *et al*., 2006). The subfamily Phasiinae is known as specialist to comprise species which attack true bugs (Heteroptera) (Arnaud, 1978). Blaschke *et al.* (2019) reported that phasiines are parasitize assortment of species from miniature discolored plant bugs (*Lygus* Hahn spp.) to leaf– footed bugs (*Leptoglossus* Guérin – Méneville spp.) including several important invasive agricultural pests as *Nezara viridula* L., *Halyomorpha halys* Stål and *Megacopta cribraria* Fabricius. Adults of Phsiinae mostly are nectar feeders. Larvae overwinter within the host bug and emerge in the following late spring or early summer (Worthley, 1924).

In Egypt, the subfamily Phasiinae is known as comprising only seven species in three tribes, namely, Cylindromyiini, Leucostomatini, and Phasiini (El-Hawagry, 2018). The first detected and Identified the species of *C. rufipes* by (Ayman and Hany, 2010), there are 3 specimens as source material which found in Ministry of Agriculture Collection, Plant Protection Research Institute, Taxonomy Department, these specimens collected from Mansouriah. by one method (Sweeping net) at 28-08-1935, from which 2 females and 1 male, in addition to the specimens were

collected recently from Western desert (El-Kharga Oasis) by sweeping net at 80-2007. The species *C. rufipes*, since that date, which was recorded in Egypt, has not been reported by anyone as a parasite on bug specially *N. millierei* .

Therefore, the present work records for the first time the presence of tachinidae fly *C. rufipes* as a facultative endoparasitoid of stink bug *N. millierei* from Egypt and includes a description of its morphology and biology .

Materials and methods

1. Collected sample of insect:

During the gathering of the green bug *N. millierei* were different methods were used to collect insects, by hand, a sweeping net or sheet-screen, (Sheet screen traps are used prior to collection to determine which types of bugs are most prevalent) in the month of October from the soya bean plant *Glycine max* from the farm of the Faculty of Agriculture at Cairo University in order to raise it to do some experiments on entomopathogenic fungi on it, where more green bugs *N. millierei* are done to do experiments to combat the fungi that pathogen to insects on that bug.

2. Rearing of green bug *Nezara millierei*:

Collected adult were maintained on plastic cage (30X12 cm) and covered with muslin. Fresh green beans *Phaseolus vulgaris* L. were supplemented for green bug feeding, which renewed every two day intervals. Sheets papers were added to lay eggs and the deposited egg-masses were daily collected and placed in petri-dish containing a pieces of moistened cotton wool and supplemented with fresh green beans until hatching. After hatching Nymphs were transferred to another rearing box. Adult were collected and put in new rearing boxes for breeding (Francati *et al*., 2019).

3. Rearing of *Cylindromyia rufipes*:

We noticed that some of the green bugs With little activity, they were isolated in empty breeding cages and monitored daily, and after 6 days, pupa was seen sticking to the green bug from the back and on the rearing cages. Pupae were collected individually in plastic cups 5cm in diameter supplement with sawdust at 25±2 ºC and 16:8h (l.:D) photoperiod. Giangiuliani and Farinelli (1995) indicate that in order to be able pupae of *Trichopoda pennis* (Phasiini) to emerge into adults they must retain in high humidity. Whereas, , in this study, we found that all pupae were dying when we used this process, so we put them in sawdust. Adult flies were collected after 2 weeks and maintained in rearing cages which supplemented with a hanged cotton ball soaked with honey solution (10%) for their feeding. Some of the flies emerged send to the Department of Insect survey and Classification Research at the Plant Protection Research Institute at the Agricultural Research Center, to scientific examination. Adult of stink bugs *N. millierei* were exposed to parasitoid cages and check daily for collection when they had parasitoid egg. Exposed bugs were transferred to the rearing cage as previous methods and notes daily until they die and collected parasite puparium (Francati *et al*., 2019). The collected adult flies emerged were taken again to the Survey and Classification Research Department of the Plant Protection Research Institute to confirm the identification, and after a scientific examination of the flies, make sure that they are of the same type that was defined before. The parasitoid adults, and the parasitized hosts were photographed. The drawings were made directly from specimens by using USB digital Microscope and original binuclear microscope by (25X measure). Four adult individuals (two

males and two females) of *C. rufipes* were deposited in the collection of the Survey and Classification Research Department, (first floor, room No. 36A) of the Plant Protection Research Institute Ministry of Agriculture Collection, Dokki, Giza.

Results and discussion

1.Taxonomy of *Nezara millierei* (Figure 1) :

Confirmation specimens : (2 males and 1 female) as a source material collected at 28-08-1935 from Mansouriah, and other specimens (3 males and 1 female) were collected from Western Desert (El-Kharga Oasis) at 80-2007.

Synonym:

Acrosternum millierei (Mulsant and Rey, 1866)

This genus *Acrosternum* has had a confusing taxonomic history. Many of the species were described or placed in the genus *Nezara* at one time or another. Also, at one time, many more species were included in *Acrosternum*; these were transferred to *Nezara*, then moved back to *Acrosternum*, and more recently have been moved back to *Nezara.* In general, species of *Acrosternum* are smaller, more pale green in color, and usually occur in dryer, more arid regions; *Nezara* species tend to be larger, more bright or vivid green, and they usually occur in more tropical or temperate areas. All pentatomids have 5-segmented antennae, and 3 tarsal segments on each foot. They generally have a large triangular scutellum in the center of the back. The body shape of adult pentatomids is generally "shieldlike," when viewed from above, but this varies between species, and is not true for the immature nymphal stages. The forewings of stink bugs are called hemelytra, with the basal half thickened while the apex is membranous. At rest, the wings are laid across the back of the insect, with the membranous wingtips overlapping. The hindwings are entirely membranous (Figure 1).

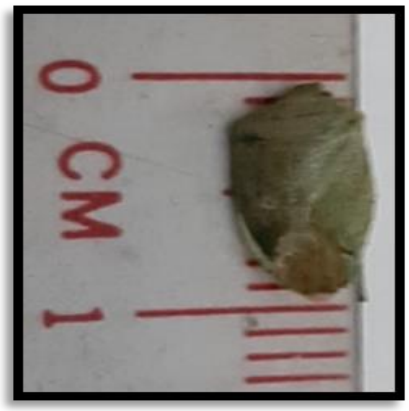

Figure (1): Adult of *Nezara millierei*

2. Taxonomy of *Cylindromyia rufipes*:

Subfamily: Phasiinae

Tribe: Cylindromyiini

Genus: *Cylindromyia* Meigen

Cylindromyia Meigen, 1803: 279. Type species: *Musca brassicaria* Fabricius, 1775, by monotypy.

Ocyptera Latreille, 1804: 195. Type species: *Musca brassicaria* Fabricius, 1775, by subsequent designation of Curtis (1837: 629).

Exogaster Rondani, 1856: 78. Type species: *Exogaster carinatus* Rondani, 1856 (= *Ocyptera*

rufifrons Loew, 1844), by original designation.
Ocypterula Rondani, 1856: 78. Type species: *Ocyptera pusilla* Meigen, 1824, by original designation.
Plesiocyptera Brauer and Bergenstamm, 1893: 56. Type species: *Ocyptera bicolor* Wiedemann, 1819, by monotypy.
Conopisoma Speiser, 1910: 144. Type species: *Conopisoma miraculum* Speiser, 1910, by original designation.
Formicocyptera Townsend, 1933: 451. Type species: *Ocyptera atrata* Fabricius, 1805, by original designation.

Diagnosis:

The genus *Cylindromyia* differs from other Phasiinae genera by combination of the following characters: Palpus strongly reduced or absent; lower facial margin visible in lateral view; prementum at least 4 times as long as wide; parafacial bare; ocellar setae proclinate; occiput with white setulae, sometimes with a few dorsal black setulae. Scutellum with 1–3 pairs of marginal setae; postmetacoxal area sclerotized. Wing cell r4+5 closed; second costal section bare ventrally. Preapical anterodorsal seta on fore tibia visibly longer than preapical dorsal seta; preapical posteroventral seta on hind tibia present. Abdominal sternites concealed by margins of abdominal tergites; tergite and sternite 7 . Female fused as a hook-like complex

***Cylindromyia rufipes* (Meigen, 1824) (Figure 2)**

Ocyptera rufipes Meigen, 1824: 215. Lectotype male (MNHN). France.

Distribution: AF: U.A. Emirates. OR: India, Pakistan. PA: Egypt, all Europe (except British Is., Scand.), Pakistan, Russia (W. Russia), Saudi Arabia, Transcaucasia, Ukraine.

Egyptian localities: Mansouriah, Western Desert (El-Kharga Oasis) and Giza.

Dates of collection: October to December.

Diagnosis:

Head: Surrounded by postocular row of setae; compound eyes bare, not hairy, they occupy a big part of the head, they are similar in both sexes; Antenna characterized all species of genus Cylindromyia which elongate in forms and flattened where the third segment is truncate apically, about 5 times as long as the second provided with numerous sensorial, the first and second segment is short, the arista very long, thickened apically and tapering toward the edge (Whip-like), the antennae situated in a grove, concave in form is a frons which surrounded by a facial edge, the later have a series of few small bristles extending on either side of the face external to the antennae and above the vibrissa, there are 7 bristles in row on each side on the frontal stripe, descending to the base of the antennae (Figure 3).

Figure (2): Adult of *Cylindromyia rufipes*

Figure (3): Head of *Cylindromyia rufipes*

Thorax: Proepisternum bare; scutellum black, with 3 pairs of marginal setae; posterior supra-alar seta present; katepisternum with 2 setae; basicosta yellowish-brown; legs dark yellow; mid tibia with 2 anterodorsal setae; hind tibia without posteroventral setae; hind femur and tibia with long setulae in posterior and ventral portion; wings, basicosta brown, bend of m narrowly rounded obtuse, the apical cross-vein more or less sinuate behind the bend, forming an obtuse angle with m, R2+3 and R4+5 nearly straight, R4+5 up curved toward Sc margin M1+2 not completed wich disappeared before ending of Sc, basal node of R4+5 branches at the distance of reaching Sc with costa (Figure 4) .

Abdomin: Long and narrow, subcylindrical or slightly clavate, constricted at the base, the terminalia of males and females not forcipate but large and doubled back on ventral surface of abdomen, there are two white rings surrounding the abdominal tergite at 3th and 5th segments, all of tergites carry a two strong setae (Figures 5 and 6).

Material examined: (1 male and 2 females) 16-10-2018 Giza; (3 Males) 03-11-2018 Giza.

The female glues at least one whitish plano-convex, -un brooded eggs on to the host body of the adult, or occasionally on late nymphal instar of the bug, and attach these eggs firmly to the body wall. The majority of the eggs are found on the sides of the thorax. Each female produces several hundred eggs during its lifespan. After hatching, newborn larva bores the host bug tegument entering its body and feeds on its body fluids for about 2 weeks. The host bug usually dies after the infection, and the larvae develop through 3 instars. Then, mature larvae emerge through its anal extremity and enter the upper soil layer to form a dark-brownish puparium (Worthley, 1924). Pupation usually requires about 2–4 weeks (Figure 7).

Figure (4): Thorax of *Cylindromyia rufipes*

Figure (5): Abdomin of ***Cylindromyia rufipes***

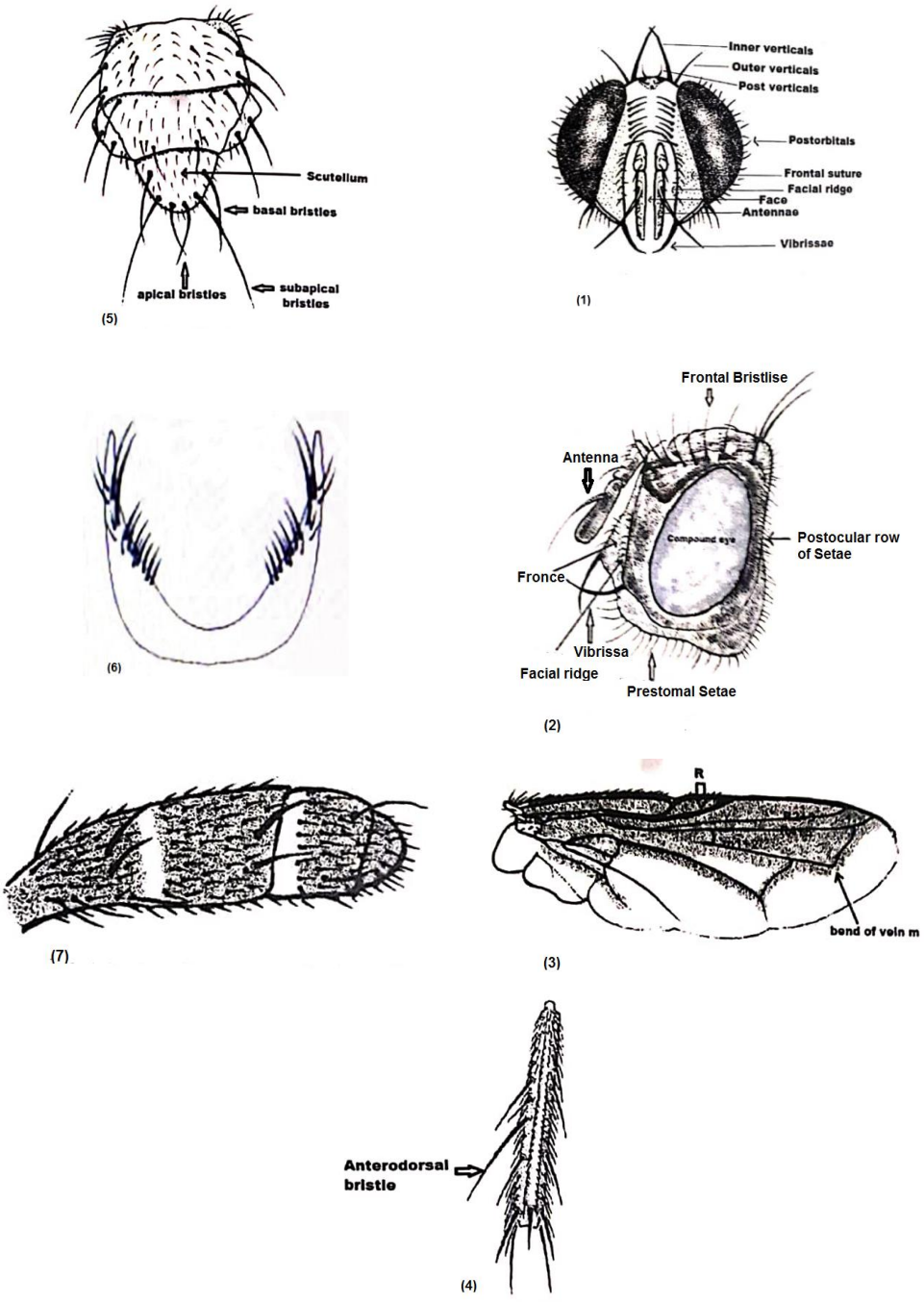

Figure (6): ***Cylindromyia rufipes*** **: (1) Head capsule, Anterior view. (2) Head capsule, lateral view. (3) Wing veination. (4) Mid tibia. (5) Thorax, dorsal view. (6) Male sternite. (7) Dorsolateral view of abdominal segments.**

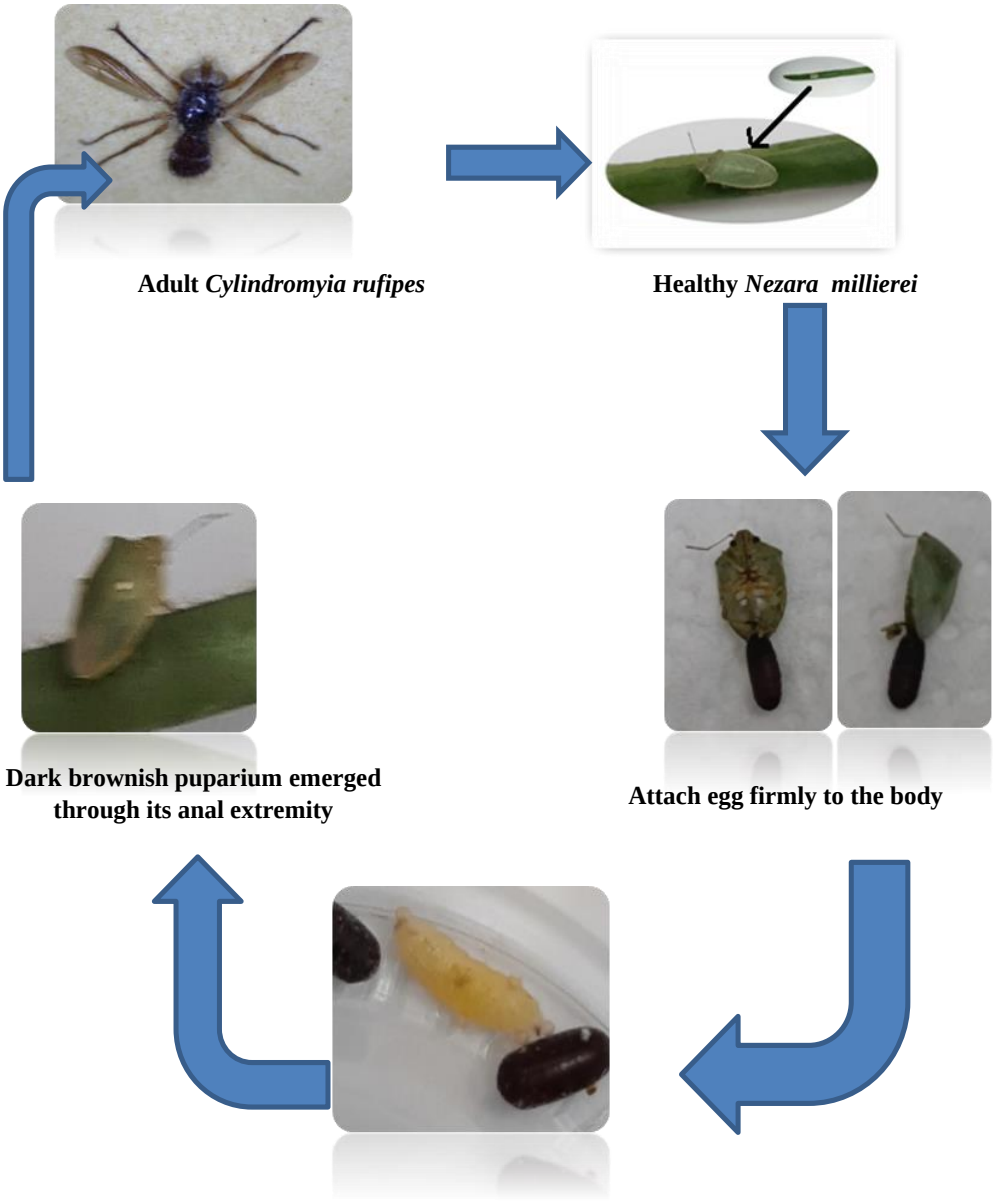

Figure (7): Life cycle of *Cylindromyia rufipes* as endoparasite of *Nezara millierei*

Our resulting agreement with other studies in *Trichopoda pennipes* (Tachinidae: Phasiinae) which have the same lifespan periods (Worthley, 1924; Salerno *et al*., 2002; Cargnus *et al*., 2011; Pétremand *et al*., 2015 and El-Hawagry *et al.* , 2020).Several stink bugs and shield bugs are considered agricultural pests, because they can grow into large populations that feed on crops, damaging production, and they are resistant to many pesticides. They are a threat to cotton, corn, sorghum, soybeans, native and ornamental trees, shrubs, vines, weeds, and many cultivated crops. Though that the effective means of reducing or mitigating green bugs *N. millierei* pests effects through the use of natural enemies as *C. rufipes* .

From the beginning of this century, tachinid flies have been involved in many operations of applied biological control against different insect pests. A review is done about utilization of Tachinidae all over the world. These parasitoid insects were used by inoculative, augmentative or inundative releases. Most of the operations were accomplished in the nearctic and neotropical regions. Sometimes great successes were obtained especially in North America and in tropical areas. Success or failure of release processes may be explained by different ways shortly analyzed.

Egypt, as a part of the Great Desert Belt, is characterized by a warm and almost rainless climate and has a greater affiliation to the Palearctic region except for the Gebel Elba, its southeastern triangle, which has a greater affiliation to the Afrotropical region (El-Hawagry and Gilbert, 2014; El-Hawagry, 2017 and El- Hawagry *et al.,* 2020). However, and according to the present study *C. rufipes* seems to be established in Egypt.

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