

## REVIEW

## The stink bug, *Andrallus spinidens* (Hemiptera: Pentatomidae), a potential predator for effective biological control

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### Abstract

The aim of this review is to describe ecological and physiological features of *Andrallus spinidens* Fabricius and to discuss various possibilities of using it as an appropriate bio-control agent in different agroecosystems. This hemipteran is a cosmopolitan predator of caterpillar pests of rice, wheat, soybean, moong, pigeon pea, maize, sugarcane and cowpea with special feeding on *Chilo suppressalis* Walker, *Naranga aenescnes* Moore, *Helicoverpa armigera* Hübner and *Spodoptera litura* Fabricius. Climate, spatial distribution of prey and type of agricultural crop are among the factors influencing the biology and spatial-temporal distribution of *A. spinidens*. Studies have shown random or aggregated distribution of the predatory bug with population peaks in April, July and October. The 1st instar nymphs have no feeding, the 2nd and 3rd (the first 2 days) instars are seedling feeders while they are voracious predators of caterpillars from the middle of 3rd instar to adulthood. The salivary gland consisted of two anterior-, two lateral- and two posterior lobes with major secretion of trypsin, chymotrypsin, amino- and carboxypeptidases. The alimentary canal has a four-sectioned midgut in which the third section seems to be the main place for digestive enzymes including  $\alpha$ -amylase, trypsin, chymotrypsin, elastase cathepsins B, L and D as well as carboxy- and aminopeptidases. *Andrallus spinidens* have shown compatibility with some insecticides and the entomopathogenic fungus, *Beauveria bassiana*. The predatory bug may be successfully reared in a laboratory using *Galleria mellonella* larvae as prey and both conservation and augmentation should be considered as biological control strategies against insect pests.

**Keywords:** *Andrallus spinidnes*, biological control, distribution, mass rearing, physiology

## Introduction

There are several constraints on rice production around the world including improper transportation, lack of suitable warehouses, unfavorable weather, insect pests, pathogens and weeds (Anonymous 2018). Insects and pathogens cause an average loss of 37% to rice yield that can vary between 24 to 41% regarding the production situation (Savary *et al.* 2000). Insects from different orders cause significant damage to rice plantations some of which are rice hispa, case worm, leaf folders, planthoppers, stem borers, armyworms, gall midges

and thrips (Zibae 2013). Due to high insect diversity in paddy fields and the need to produce healthy rice, it is recommended to design an efficient system of integrated pest management to alleviate pest population outbreaks. Some steps should be included in the system like proper cleaning of agronomical equipment, using clean seeds and varieties resistant to environmental extremes and insect damage, balanced use of fertilizers, conservation of natural biocontrol agents, use of eco-friendly pesticides and efficient storage of harvested

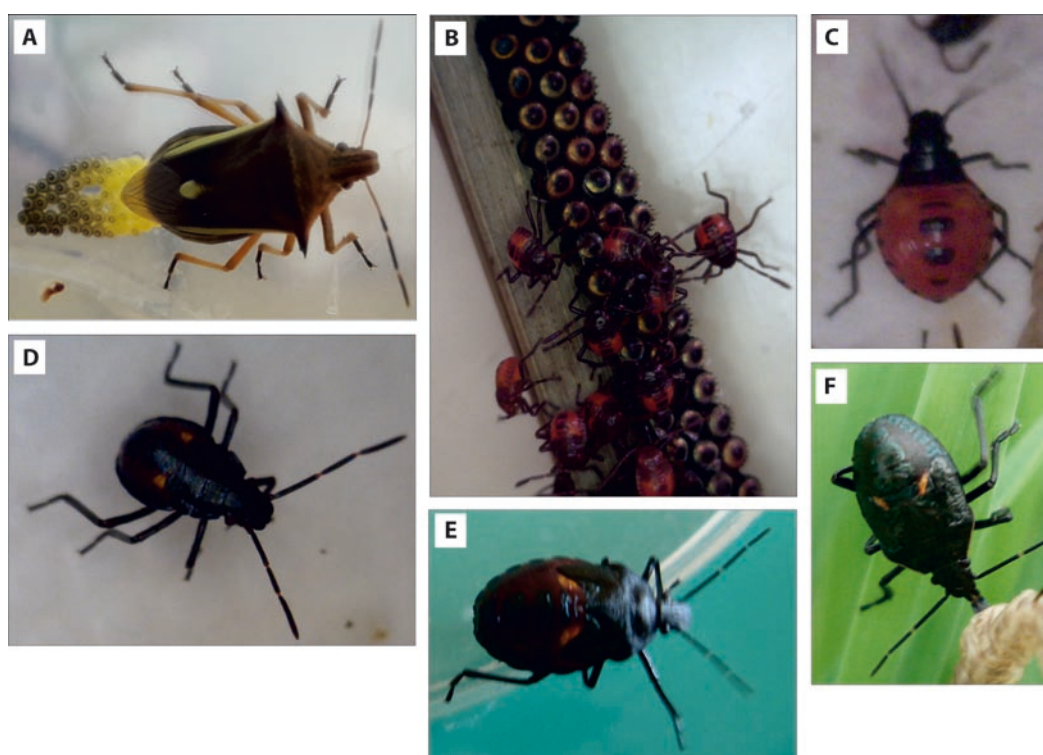
grains (Reissig *et al.* 1986). Due to the urgent need for an increased food supply which involves reducing rice losses by pests, farmers neglect integrated pest management and freely spray paddy fields with different classes of insecticides even higher than the recommended doses (Parween and Nakagoshi 2001). Although such action may be effective in short-term periods, it will definitely lead to extensive problems including disruption of an ecological balance between pests and predators, environmental pollution, products containing pesticide residue and pesticide resistance (Parween and Nakagoshi 2001; Zibae *et al.* 2009; Zibae 2019). The afore-mentioned concerns have forced producers and consumers toward using eco-friendly pest control methods, mainly biological control. Microbial bacteria like *Bacillus thuringiensis* and fungi like *Beauveria bassiana* and *Metarhizium anisoplia*, parasitoids of Braconids and Trichogrammatids in addition to predators like *Andrallus spinidens* Fabricius (Hemiptera: Pentatomidae) are among the major biocontrol agents of rice pests around the world (Ramzi and Zibae 2014; Bhojendra *et al.* 2019). Their distribution, efficiency and adaptation to cultivation systems should be determined for potential use in conservation or augmentation strategies against rice pests.

*Andrallus spinidens* is a predatory bug of caterpillars that naturally resides in paddy fields. Apart from other biocontrol agents like parasitoids, conservation

strategies by providing proper habitat, using fewer toxic pesticides and providing seedlings in winter increase its population which naturally suppresses outbreaks of rice caterpillars. *Andrallus spinidens* belongs to the Pentatomid family of Hemipterans which was identified by Fabricius while Distant in 1902 reported it from India and described its current morphological characteristics (Distant 1902). In the current review, the biological and physiological characteristics of *A. spinidens* are discussed along with its potential compatibility with other control methods.

## Morphological identification

Eggs are oval-shaped with 1.149 mm length and 0.84 mm diameter. The top of each egg is covered with a disc-shaped valve and a dark brown ring. The eggs are white to pale yellow just after laying but turn dark with embryonic growth (Rajendra and Patel 1971; Javadi *et al.* 2005) (Fig. 1B). The newly hatched nymphs are bright red and turn dark red in a matter of hours (Fig. 1B). In the second nymphal stage, there are six black spots on the lateral sides of the abdomen and three black spots on the center of the abdomen (Fig. 1C). The third nymphs are similar to second nymphs but they are dark red (Fig. 1D). In fourth and



**Fig. 1.** Adults, eggs and nymphal instars of *Andrallus spinidens*. A – adult, B – eggs and 1st instar nymphs, C – 2nd instar nymph, D – 3rd instar nymph, E – 4th instar nymph, F – 5th instar nymph

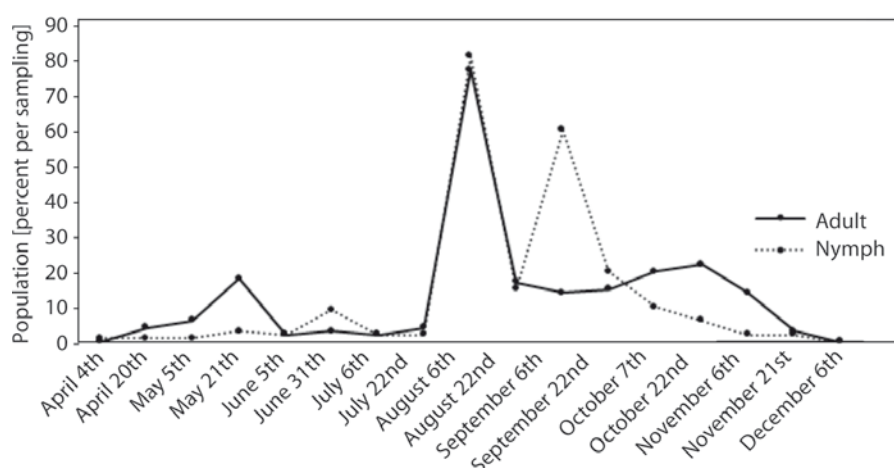
fifth nymphal stages, imaginal discs and dorsal spines are distinct. There are four black spots in the center of the abdomen and seven black spots on the lateral sides of the abdomen although some yellow spots are visible on the abdominal segments (Rajendra and Patel 1971; Javadi *et al.* 2005) (Fig. 1E). Adults (males 13.4 mm long, females 15.8 mm long) are pale brown with distinct, dense black punctuation on the head, pronotum, scutellum and hemelytra which form a distinct pattern on the head and anterior part of the pronotum. Antennae have dark brown segments with very fine setae; the rostrum is robust and long-reaching to the posterior coxae (Ghate *et al.* 2012). The head is almost rectangular with the two compound eyes on both sides and a pair of a simple red eyes near the compound eyes (Rajendra and Patel 1971). The pronotum is hexagonal and densely punctured. The scutellum is longer than wide and densely punctured with a longitudinal smooth line in the middle (Ghate *et al.* 2012) (Fig. 1A). Corium of hemelytra is broad and extends to the lateral margin of the wing with two white stripes on the outer sides of the corium wing (Javadi *et al.* 2005).

## Distribution and host range

*Andrallus spinidens* has been reported in different geographical areas of eastern Asia, Australia, North Africa, some parts of Europe and North America [as a predatory bug of caterpillars damaging several agricultural crops including *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae)], *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), *Chilo suppressalis* Walker (Lepidoptera: Crambidae) and *Naranga aeneascens* Moore (Lepidoptera: Noctuidae), *Mythimna separate*

Walker (Lepidoptera: Noctuidae), *Thermesia rubricans* Boiduval (Lepidoptera: Noctuidae), *Mythimna unipuncta* Walker (Lepidoptera: Noctuidae), *Melanitis leda* L. (Lepidoptera: Nymphalidae), *Pelopida smathias* Fabricius (Lepidoptera: Hesperidae), *Spilosoma oblique* Walker in addition to a coleopteran, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) (Lepidoptera: Erabidae) (Distant 1902; Maxwell-Lefroy 1909; Cherian and Brahmachari 1941; Rajendra and Patel 1971; Singh and Gangrade 1975; Pawar 1976; Rao and Rao 1979; Singh and Singh 1989; Pandey *et al.* 2002; Mohaghegh and Amir-Maafi 2007; Claver and Jaiswal 2013; Shylesha and Sravika 2018; Bhojendra *et al.* 2019).

Several environmental factors may affect the biology and spatial-temporal distribution of *A. spinidens* including climate, distribution of prey and type of host plant. Javadi *et al.* (2005) determined the spatial-temporal distribution of the adults and the nymphal stages of *A. spinidens* in rice fields of northern Iran on two rice varieties. These authors reported a random to aggregated population index on the two rice varieties. Moreover, they monitored population changes from March to October and reported the highest number of both adults and nymphs in July although two small peaks were recorded in May and October for adults as well as in June and September for nymphs (Javadi *et al.* 2005) (Fig. 2) (Rezvani and Shahosseini 1976). Shylesha and Sravika (2018) found active feeding of both adults and nymphs on the different larval stages of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) during the 38th and 39th standard meteorological weeks of maize growth when *S. frugiperda* caused severe damage. Bhojendra *et al.* (2019) found no significant difference in predation rate among five sampling locations with different altitudes of *A. spinidens* in northwestern Himalayas.



**Fig. 2.** Seasonal occurrence of *Andrallus spinidens* in rice field in northern Iran, Guilan province (Javadi *et al.* 2005)

## Biology

The females of *A. spinidens* live longer than males, but it may vary between mated and unmated individuals. Bhojendra *et al.* (2019) reported a longevity of 12–13 days for unmated males and 17–19 days for unmated females. In another study, the unmated males and females which fed on *Rivulia* sp. larvae survived for 24 and 32 days compared to 10 and 15 days in mated males and females, respectively (Singh and Singh 1989). Therefore, it may be inferred that sex, virginity, and prey are factors affecting the longevity of *A. spinidens*. Adults mate several times during their life although they are not able to mate right after emergence since it takes 5–16 days for sexual maturity (Rezvani and Shahosseini 1976). Three days after mating, females lay eggs on rice leaves or stems in patches of 2–4 rows, 3–5 cm long, which stick to leaves with a viscous material (Fig. 1A). Each female may lay 2–6 patches containing 7–96 eggs (Rezvani and Shahosseini 1976; Shanker *et al.* 2017) depending on host prey and climate both in the field and laboratory (Rezvani and Shahosseini 1976; Manley 1982; Singh and Singh 1989).

The period of embryonic growth in *A. spinidens* has been reported as 7.63 days at 26.6°C (Rajendra and Patel 1971), 6.6 days under field conditions (Singh and Singh 1989), 9 days at 26.6°C (Javadi *et al.* 2005), 6–10 days at 25°C (Shanker *et al.* 2017) and 6.41–6.77 days at four locations of northwestern Himalayan regions (Bhojendra *et al.* 2019). Shintani *et al.* (2010) indicated the temperature of 16.5°C and 102.9 degree-days as the developmental zero and thermal constant of the eggs. Manley (1982) reported five nymphal instars in *A. spinidens* and the total nymphal period of 18.54 days fed *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). The total nymphal period of *A. spinidens* has been shown to be 24 and 26.33 days once *N. aenesenes* and *C. suppressalis* were used as the preys. The detailed nymphal durations were 3.5 days for 1st nymphs, 4.33 and 4.5 days for 2nd nymphs, 4.16 and 4.66 for 3rd nymphs, 4.5 and 5.66 days for 4th nymphs, 7.5 and 8.16 days for 5th nymphs fed *N. aenesenes* and *C. suppressalis*, respectively (Javadi *et al.* 2005). Khodaverdi *et al.* (2012) reported a total nymphal period of 26.12 days in *A. spinidens* at 25°C fed *S. littoralis*. Bhojendra *et al.* (2019) demonstrated that the 1st and the 5th instar nymphs had the shortest (2.67 days) and the longest (5 days) nymphal periods in the five populations of *A. spinidens* although there were no significant differences between the populations.

Khodaverdi *et al.* (2012) reported the demographic parameters of *A. spinidens* fed *S. littoralis* at 25°C based on the periods of all developmental and adulthood stages while considering both sexes. No mortality was

recorded of eggs and 1st nymphal stages while adult survival decreased along with aging. Overall, the mortality rate was significantly lower at nymphal stages than adulthood. The highest life expectancy ( $e_x$ ) was found for eggs (67 days) then it gradually decreased to adult. The Gross-( $GRR$ ) and net-( $R_0$ ) reproductive rates were calculated as 192.61 female eggs and 102.77 female offspring. The intrinsic ( $r_m$ ) and finite ( $\lambda$ ) rates of increase were found to be 0.082 and 1.082 days with intrinsic rates of birth and death of 0.0894 and 0.0073, respectively (Khodaverdi *et al.* 2012).

There are two studies on the seasonal occurrence of *A. spinidens* in Iran and Japan. At first, Javadi *et al.* (2005) demonstrated that hibernated adults start to feed and to look for mates in early May. The population density increased along with temperature during spring and summer when the highest population density was recorded in the middle of August. It gradually decreased to October when the adults hibernated for winter. Three generations have been reported for *A. spinidens* in paddy fields of Iran with population fluctuations of *C. suppressalis* and *N. aenesenes* (Javadi *et al.* 2005). In a similar study, Shintani *et al.* (2010) reported the findings of biannual observations. In 2006, the sole presence of 5th instar nymphs was recorded in early to mid-July and early August while both nymphs and adults were found in mid-August which was continuous until late October. Finally, no *A. spinidens* was observed from December 2006 to March 2007. In 2007, the first incidence of adults was found in April to mid-May, then the nymphs were observed from late-May to early-June. Adults were observed again in mid-July to produce a new generation (Shintani *et al.* 2010).

## Feeding behavior and digestion

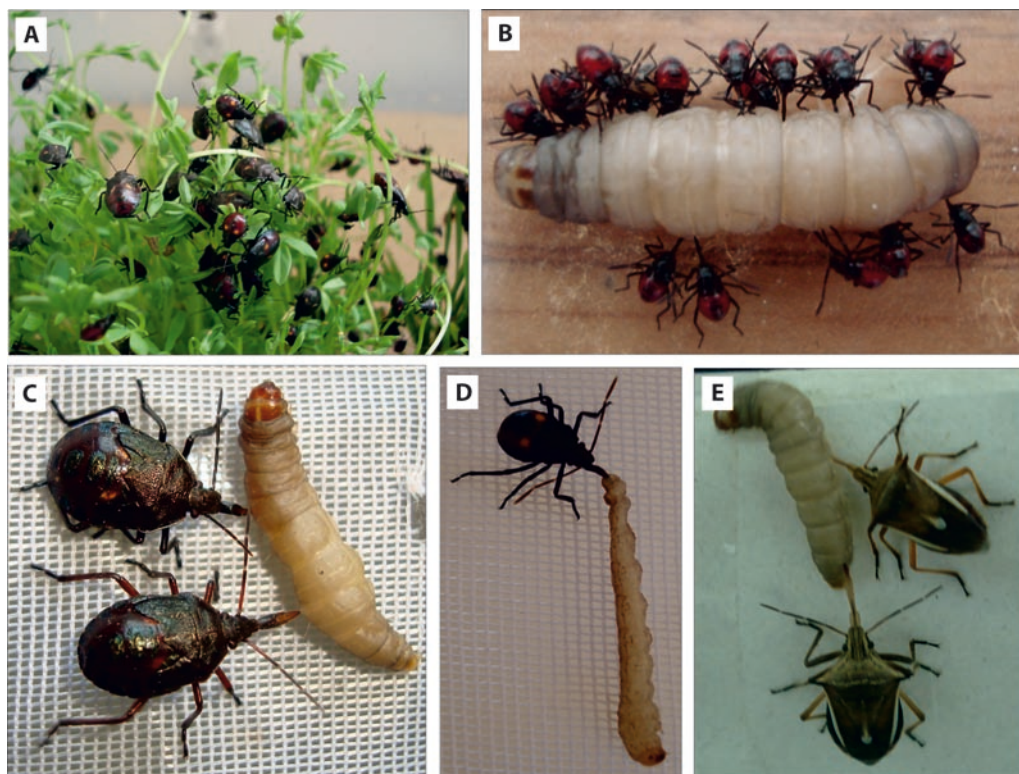
The 1st instar nymphs are aggregated around the place of egg-laying. They have almost no feeding, but some may utilize leaf sap of rice or other gramineous seedlings (Fig. 3). The 2nd instar nymphs look for prey between plants, but they may feed on seedling sap rather than host larvae (Fig. 3). From 3rd instar nymphs to adulthood, a complete predatory behavior may be obvious through pursuing prey and active feeding on caterpillars (Rajendra and Patel 1971). Nymphs and adults insert their stylets near the head or abdomen of prey, paralyze it and liquefy the body to be sucked (Sorkhabi-Abdolmaleki *et al.* 2014) (Fig. 3). Nymphs of *A. spinidens* repeatedly attack caterpillars which may take 1–4 min while the feeding period may last almost 8 h (Manley 1982).

In a series of studies, the digestive physiology of *A. spinidens* was investigated through several bio-

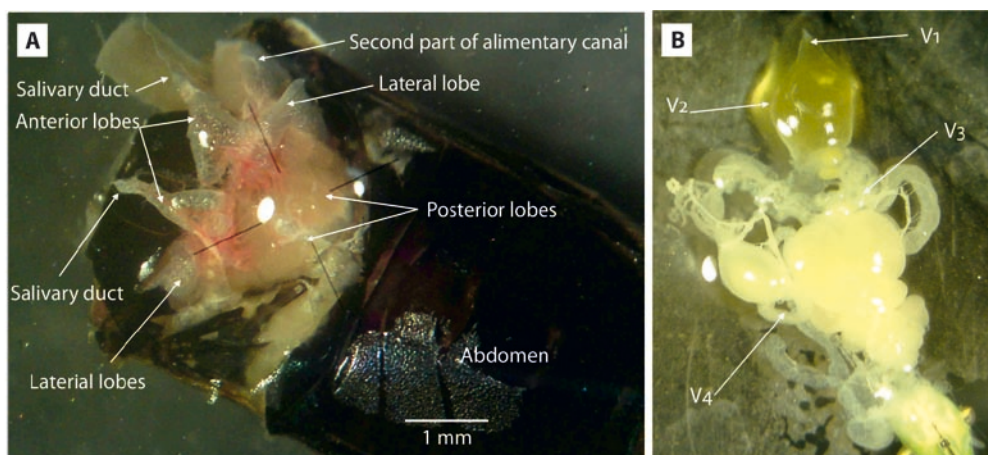


chemical and physiological processes. Zibae *et al.* (2012a) morphologically characterized salivary glands of *A. spinidens* and biochemically determined its proteolytic profile. The salivary gland consisted of two anterior-, two lateral- and two posterior lobes (Fig. 4A). Trypsin, chymotrypsin, amino- and carboxypeptidases were the major proteases in the salivary secretions. It is necessary to mention that proteases are not the sole enzymes important in extra-oral digestion

of *A. spinidens* as two reports showed the roles of  $\alpha$ -amylase and lipase in salivary secretion (Zibae *et al.* 2011; Zibae *et al.* 2012b). The alimentary canal of *A. spinidens* has a four-sectioned midgut in addition to foregut and hindgut. The third section of the midgut seems to be the main region of digestive enzyme secretion (Sorkhabi-Abdolmaleki *et al.* 2013) (Fig. 4B). Digestive  $\alpha$ -amylase, trypsin, chymotrypsin, elastase; cathepsins B, L and D proteases as well as carboxy- and



**Fig. 3.** Feeding of *Andrallus spinidens* nymphs and adults on seedlings and *Galleria mellonella*. The second instar nymphs initially feed on seedlings then they start to suck prey bodies. 3rd instars and adults solely feed on caterpillars (original images). A – feeding of first and second nymphal instars on plants, B – aggregated feeding behavior of early nymphal instars, C and E – feeding of 4th nymphal instars and adults on prey, D – feeding duration to empty body mass



**Fig. 4.** A – salivary gland, B – alimentary canal of *Andrallus spinidens* adults (V – ventriculus). Courtesy of Zibae *et al.* (2012 a); Sorkhabi-Abdolmaleki and Zibae (2013)

aminopeptidases were found in the midgut secretions of *A. spinidens* adults which were shown to use specific substrates and inhibitors (Sorkhabi-Abdolmaleki *et al.* 2013; Sorkhabi-Abdolmaleki *et al.* 2014).

The course of food processing and secretagogue mechanisms were studied in the adults of *A. spinidens* fed the larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae) as a model prey (Sorkhabi-Abdolmaleki and Zibae 2013). Overall results indicated the highest activities of digestive enzymes 12–24 h post-feeding on prey. The authors attributed the long-lasting digestion process in *A. spinidens* to long liquefaction and sucking of the body fluids of their prey (Sorkhabi-Abdolmaleki and Zibae 2013).

Additionally, Sorkhabi-Abdolmaleki and Zibae (2013) investigated the activities of digestive enzymes in *A. spinidens* feeding on *C. suppressalis*, *G. melonella*, *E. kuehniella* and *P. interpunctella* (Lepidoptera: Pyralidae) to find the most suitable prey for mass rearing. The highest biomass gained, and total protein was found in the nymphs fed *C. suppressalis* and *G. melonella*. The highest activities of carbohydrases were recorded after feeding on *C. suppressalis* and *E. kuehniella* while lipase had the highest activity on *G. melonella*. Feeding of nymphs on both *G. melonella* and *E. kuehniella* caused the highest activities of serine and cysteine proteases of *A. spinidens*. The authors concluded that *G. melonella* was the most suitable prey for mass rearing of *A. spinidens* because of higher gained weight and proper activity of digestive enzymes (Sorkhabi-Abdolmaleki and Zibae 2013).

Apart from physiological studies, functional responses of *A. spinidens* were studied to find predation characteristics against different prey densities. As a pioneer study, Manley (1982) reported that *A. spinidens* had a significant impact on the periods encountered with moderate or high prey densities (outbreak) because of a short life-cycle and continuous feeding on available caterpillars. In 2012, a demographic analysis of *A. spinidens* (nymphs and adults) predation was studied in the presence of *S. littoralis* larvae (Khodaverdi *et al.* 2012). It was found that 1st instar nymphs have no predation while the four other instars in addition to adults may consume 22, 60, 90, 146, 1116, 1140 larvae during 11, 15, 15, 17, 93 and 95 days, respectively. Demographic analysis of predation revealed an elevated predation rate from the ages 12 to 23 days, but it slightly decreased at ages 24 to 27 days because of mortality in the developmental stages. Afterwards, the predation rate again increased at 4th and 5th instar nymphs. Moreover, the authors recorded that 4.15 larvae of *S. littoralis* were required for the reproduction of *A. spinidens* (Khodaverdi *et al.* 2012). In a similar study, Shanker *et al.* (2017) determined the functional response of *A. spinidens* to *S. mauritia* as the type II

curvilinear functional response along with the higher number of exposed prey. Such a response demonstrates that the predatory bug kills more individuals at a higher density of prey, with a constant search rate and saturation of predator (Shanker *et al.* 2017).

Hyodo *et al.* (2014) designed a study to find the costs and benefits of group predation in *A. spinidens*. The authors believed in some advantages of group predation including the promotion of foraging efficiency, size of the prey and hunting success. They found that the number of attacks by nymphs of *A. spinidens* both individually and in four groups against 6th instar larvae of *S. litura* were significantly higher than 3rd and 4th larval instars. The authors defined the hunting time from touch and paralysis. They reported a longer hunting time toward 6th instar larvae than 3rd and 4th instars. Also, the individual predatory bugs had longer hunting times than four predator groups and that the latter had a higher predation success against third and 4th instar larvae (Hyodo *et al.* 2014). Taken collectively, the authors concluded that group predation has an advantage towards large prey mainly for early nymphs because of their smaller size and narrow host range which has been similarly observed in field observations (Manley 1982).

## Compatibility with other pest control methods

Production of sufficient food for the increasing population of humans is a desirable objective in several cropping systems but it should be kept in mind that the health of agricultural products is a critical issue mainly due to pesticide residues. Because of diversity and economic importance of rice pests, chemical spraying is inevitable in several cases, but such an action has its own consequences of adversely affecting survival. The fitness of natural enemies is an important issue in integrated pest management. Therefore, it is mandatory to conduct screening studies to discriminate between pesticides with low toxicity against natural enemies and those which have acute toxicity against both insect pests and biocontrol agents.

Gholamzadeh-Chitgar and colleagues designed a series of studies to determine the compatibility of *A. spinidens* with conventional insecticides sprayed on rice fields and the entomopathogenic fungus *Beauveria bassiana* Vuillemin (Gholamzadeh-Chitgar *et al.* 2014, 2015, 2016, 2017). The toxicity of diazinon, fenitrothion, and chlorpyrifos were compared to 5th instar nymphs of *A. spinidens* with LC<sub>50</sub> values of 1772.79, 380 and 449.3 ppm, respectively. It was inferred that fenitrothion was the most toxic insecticide on the nymphs while diazinon showed the least toxicity based on LC<sub>50</sub>



(Gholamzadeh-Chitgar *et al.* 2015). Sublethal concentrations of the insecticides significantly changed some ecological and physiological parameters of the treated *A. spinidens*. Fecundity, egg hatchability and longevity of males significantly decreased following insecticide treatments compared to the control. The pre-ovipositional period significantly increased and no significant differences were observed in female longevity. In all evaluated parameters, fenitrothion had the most significant effects compared to diazinon and chlorpyrifos (Gholamzadeh-Chitgar *et al.* 2015). Moreover, the insecticide treatments on *A. spinidens* alleviated the intrinsic rate of population increase, finite rate of population increase, and gross reproduction rate compared to the control. For that, fenitrothion had the most effects on these parameters. Analysis of the age-specific survival rate and the age-specific number of progeny per day indicated a significantly lower survival rate in the treated individuals. The insecticides made changes in cellular energy allocation of *A. spinidens* (Gholamzadeh-Chitgar *et al.* 2014). Although no changes were reported in the amounts of total carbohydrates and glycogen within the treated nymphs, the amount of total protein increased in the treated nymphs compared to the control mainly following treatment with chlorpyrifos. The nymphs treated with all insecticides showed no statistical difference in the total energy content compared to the control (Gholamzadeh-Chitgar *et al.* 2014). In addition, sublethal concentration of the entomopathogenic fungus, *B. bassiana*, was investigated on the demography and some physiological parameters of *A. spinidens*. The  $LC_{50}$  concentration of  $3 \times 10^5$  conidia  $\cdot$  ml<sup>-1</sup> showed no significant differences in the pre-oviposition period, fecundity, egg hatchability, the longevity of females and males as well as life table parameters. Also, no significant differences were found in the amounts of total lipid, carbohydrate and protein as well as the content of energy between treated nymphs by *B. bassiana* and the control ones (Gholamzadeh-Chitgar *et al.* 2016, 2017).

Virulence of the two isolates of *B. bassiana* and immune responses of *A. spinidens* were determined in two studies by Firouzbakht *et al.* (2015, 2016). The  $LC_{50}$  concentrations of the isolates BB<sub>2</sub> and Am-118 were calculated as  $37 \times 10^5$  and  $15 \times 10^3$  conidia  $\cdot$  ml<sup>-1</sup>. Moreover, the higher activities of chitinase, lipase, phosphatase and trypsin-like protease were reported in the isolate AM-118 than in BB<sub>2</sub>. The authors concluded that AM-118 is more virulent against *A. spinidens* although both isolates caused significant mortality in *C. suppressalis*, the major pest of rice and host of *A. spinidens* (Firouzbakht *et al.* 2015). These authors reported four types of hemocytes in the hemolymph of *A. spinidens* including prohemocytes, plasmatocytes, granulocytes and oenocytoids which showed changes

in their numbers upon injection by both fungal isolates. In detail, the numbers of total and differentiate hemocyte counts altered following isolate injection at different time intervals in addition to phenoloxidase activity. These results concurred with earlier findings on different virulence of BB<sub>2</sub> and AM-118 against *A. spinidens* (Firouzbakht *et al.* 2016).

## Mass rearing

Mass rearing of biocontrol agents is the fundamental step to design a successful biological control program to ensure accessibility of the desirable numbers of individuals to be used in agroecosystems. Earlier studies revealed successful rearing of *A. spinidens* on several hosts including *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), *E. kuehniella* and *S. litura* (Mohaghegh and Amir-Maafi 2007; Shanker *et al.* 2017).

In this section, steps for proper mass rearing of *A. spinidens* are presented which initially were suggested by De Clercq and Degheele (1993) but we slightly modified them after several experiments. This method requires the larvae of *G. mellonella* to be a suitable host for both feeding and reproduction of *A. spinidens*. The larvae of wax moths may be reared on an artificial diet containing 400 g honey, 80 g wax, 328 ml glycerin, 800 g wheat flour and 200 g yeast. The materials should be incorporated together as a paste. The males and females of moths are transferred to a funnel-like apparatus that has been sealed with a filter paper as a site of oviposition. The eggs are gently collected and transferred to the paste. The larvae of wax moths are reared at  $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  of relative humidity in darkness. The fourth and fifth instar larvae are used to feed *A. spinidens*. The collected eggs of *A. spinidens* from rice fields are put in a container (13  $\times$  10 cm) with cotton soaked in distilled water. It is recommended to leave 1st instar nymphs intact because of the high rate of mortality but they should be transferred to bigger containers provided with both wet cotton and pots of rice seedlings. In this developmental stage to the initial days of 3rd instars, nymphs of *A. spinidens* rarely feed on the host and they prefer sap-sucking on rice seedlings. In the 48–96 h post-third instar, they should be provided with the larvae of *G. mellonella*. The predatory bug may be reared during the entire cropping season if the following points are kept in mind (Ebadi and Ghaninia 2003): the presence of host plants in rearing containers as the stimulator of feeding and mating, the supply of enough food and space to prevent cannibalism, and keeping the sex ratio of two males per one female. Mohaghegh and Amir-Maafi (2007) compared the effect of live

and frozen larvae of *G. mellonella* and *E. kuehniella* on the reproduction of *A. spinidens*. There were no significant differences in development and weight between the individuals fed both live and frozen larvae of *G. mellonella* while the predatory bug fed live larvae of *E. kuehniella* had a higher weight than those fed frozen prey. The longevity and fecundity of *A. spinidens* showed no significant differences between two types of prey while the egg hatchability decreased in the individuals fed frozen prey. The authors concluded that frozen prey may be suitable as the feeding source in the case of a shortage of live larvae.

## Conclusions

The use of biological control agents as a new, low-cost, efficient and risk-free approach has become more and more common among experts, researchers, and authorities. This is a critical issue because of the necessity to produce sufficient and healthy foodstuffs for the overgrowing population of humans. Rice, as a strategic product and as one of the most important human food sources, is no exception. Given the increasing use of different pesticides to keep insect pests and pathogenic diseases away from crops, it should be taken into account that excessive use of chemicals influences the normal biological performance of beneficial organisms, and more importantly the environment of which we are a part. Therefore, we need a safe approach in which biocontrol agents are the most suitable candidates for it. Such characteristics as short life span, voracious feeding on prey and appropriate reproduction make *A. spinidens* a reliable biocontrol agent of caterpillars in several cropping systems mainly rice. Several studies imply the natural occurrence of *A. spinidens* in agroecosystems so the first steps should be concentrated on conservation of the population of the predatory bug in fields. Preserving plants around fields, the use of low-risk insecticides or other biological control agents such as entomopathogenic fungi, are the most important ways to keep the native population of *A. spinidens*. Due to population fluctuations of target caterpillars, mainly *C. suppressalis* and *Spodoptera* spp., it is recommended to keep a laboratory stock of *A. spinidens* to be released in the infested rice through augmentation techniques which will lead to desirable control of pest density. Considering the different ecological and physiological characteristics of *A. spinidens* in terms of feeding on different hosts and adaptation to other control methods, it is necessary to develop a precise and regular biological pest control program using this predatory bug in order to gain healthy produce with minimal economic and biological costs.

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