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Research Article

Developmental biology, laboratory rearing, and predatory potential of *Andrallus spinidens* (F.) (Hemiptera: Pentatomidae)

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ABSTRACT: *Andrallus spinidens* (F.) (Hemiptera: Pentatomidae: Asopinae) is a native generalist predator with significant potential as a biological control agent against lepidopteran pests in tropical agroecosystems. Despite documented field co-occurrence with the invasive fall armyworm, *Spodoptera frugiperda*, no prior study had simultaneously characterised the complete developmental biology, fecundity, and adult longevity of *A. spinidens* reared exclusively on this prey. The present study addresses this gap under controlled conditions of 30 ± 2°C and 75 ± 5% relative humidity (RH). The species completed five nymphal instars with a mean total nymphal period of 19.33 ± 0.58 days and egg incubation of 6.33 ± 0.58 days, with 95-100% hatching success. The fifth instar was the longest (6.33 ± 0.58 days). Total egg-to-adult development averaged 40.33 ± 0.58 days, and adult longevity averaged 14.67 ± 1.53 days. Females produced 2-6 egg batches with lifetime fecundity of 25-70 eggs. A standardised rearing protocol using green bean pods for first instars and size-matched *S. frugiperda* larvae for subsequent instars was established. These findings provide the first prey-specific biological dataset to support deployment of *A. spinidens* in augmentative biocontrol programs against fall armyworm in Indian maize agroecosystems.

KEYWORDS: *Andrallus spinidens*, biological control, developmental biology, fecundity, laboratory rearing, *Spodoptera frugiperda*

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INTRODUCTION

Andrallus spinidens (F.) (Hemiptera: Pentatomidae: Asopinae) is a widely distributed predatory stink bug that has long attracted attention as a promising native biological control agent across tropical and subtropical farming landscapes. As a generalist predator, it preys on several economically important lepidopteran pests of rice, wheat, soybean, maize, sugarcane, and cowpea, including *Chilo suppressalis* Walker, *Naranga aenescens* Moore, *Helicoverpa armigera* Hubner, and *Spodoptera litura* Fabricius, and its biocontrol potential has been documented in rice fields across India, Malaysia, and Iran (Gholamzadeh-Chitgar *et al.,* 2020). Field surveys have confirmed its presence across a broad range of Indian crops including wheat, maize, pigeonpea, gram, rice, beans, and cowpea (Maurya *et al.,* 2020), and it figures among the most widely distributed predatory Asopinae recorded from the Indian subcontinent (Maheshwari *et al.,* 2023). The bug completes three to four generations each year, reaching peak populations between September and December (Shintani *et al.,* 2010), and shows reasonable compatibility with select insecticides and the entomopathogenic fungus *Beauveria bassiana*, making it amenable to integration within IPM programs (Gholamzadeh-Chitgar *et al.,* 2020).

That said, the biological knowledge base for *A. spinidens* remains surprisingly incomplete. Earlier laboratory investigations examined its development and predatory performance using *S. litura* (Khodaverdi *et al.,* 2012; Uematsu, 2006), *Spodoptera mauritia* Boisduval

(Shanker *et al.,* 2017), and factitious prey such as *Plodia interpunctella* Hübner, *Galleria mellonella* L., and *Ephestia kuehniella* Zeller (Ebadi & Ghaninia, 2003; Mohaghegh & Amir-Maafi, 2007). While these studies laid useful groundwork, each of these prey species differs considerably from *Spodoptera frugiperda* J.E. Smith in body size, nutritional profile, and crucially, in its aggressive defensive behaviour characteristics that can meaningfully alter a predator's developmental trajectory and feeding efficiency. Notably, none of these prior studies evaluated the complete developmental biology, fecundity, adult longevity, and stage-wise feeding potential of *A. spinidens* simultaneously on a single prey system. Whether the biological parameters recorded against this prey hold true when *A. spinidens* encounters *S. frugiperda,* which is a far larger, more mobile, and defensively active noctuid larva, is a question that has simply never been tested.

This knowledge gap matters more today than ever before. Since *S. frugiperda* was first detected in Indian maize fields in 2018, it has spread with alarming speed, reaching over 90% of the country's maize-growing areas within just 16 months (Sharanabasappa *et al.,* 2018; Suby *et al.,* 2020), and has now become a serious threat to food security. In India, yield losses due to FAW have ranged from 20% to over 45%, especially in areas where early control was not implemented (ICAR-NBAIR, 2020). What makes this situation particularly compelling from a biocontrol perspective is that *A. spinidens* has already been observed in the field, naturally preying on *S. frugiperda* larvae in organically grown maize in India (Shylesha & Sravika, 2018). This field co-occurrence is a strong ecological signal — it tells us that *A. spinidens* is already engaging with this invasive pest in real agricultural settings. Yet, no study to date has simultaneously documented the complete immature development, adult fecundity, adult longevity, and instar-specific predatory potential of *A. spinidens* reared exclusively on *S. frugiperda* under controlled conditions. In the absence of such an integrated, prey-specific biological dataset, it is impossible to assess, scale up, or recommend its use in augmentative biocontrol programs with any scientific confidence. The broader literature on Asopinae also makes clear that prey identity significantly shapes predator performance, and that prey-specific rearing protocols are essential before any natural enemy can be reliably deployed in the field (Plata-Rueda *et al.,* 2022; Pires *et al.,* 2023).

The present study was therefore designed to fill this specific and previously unaddressed gap. Unlike earlier studies that evaluated *A. spinidens* on surrogate or factitious prey, this is the first study to simultaneously document: (i) the complete developmental biology of *A. spinidens* reared on *S. frugiperda* larvae under controlled laboratory conditions; (ii) fecundity and adult longevity parameters on this ecologically relevant prey; (iii) a standardised laboratory rearing protocol using fresh *S. frugiperda* larvae; and (iv) the instar-wise predatory potential of *A. spinidens* nymphs and adults against different larval instars of *S. frugiperda*. Together, these findings provide the first comprehensive, prey-specific biological dataset needed to support the deployment of this native predatory bug as part of a science-based IPM strategy against *S. frugiperda* in Indian maize agroecosystems.

MATERIALS AND METHODS

Collection and colony establishment

Adults of the predatory stink bug *A. spinidens* (F.) were collected from wheat fields at the Norman E. Borlaug Crop Research Centre, Pantnagar, Uttarakhand, during March-April 2024 and brought to the laboratory. Ten pairs (one male and one female) were placed individually in rectangular plastic containers (20 cm × 10 cm × 10 cm) covered with muslin cloth for ventilation and allowed to mate. Males were separated immediately after mating, as mated males of *A. spinidens* have a significantly reduced postmating lifespan compared to virgins (Singh & Singh, 1989), and their continued presence risks disturbance during oviposition and data recording. Separated males were maintained individually with prey to monitor longevity.

Prey rearing and control treatment

Spodoptera frugiperda (J.E. Smith) was reared on fresh maize (*Zea mays* L.) leaves under laboratory conditions to ensure a continuous prey supply, since maize is the primary host of this pest and supports its complete development. To account for natural prey mortality independent of predation, a parallel control treatment was maintained in which *S. frugiperda* larvae were held on maize leaves under identical conditions but without the predator. Natural mortality recorded in controls was used to correct predation estimates.

Developmental biology, rearing, and data recording

Egg batches and early instar nymphs of *A. spinidens* were collected from maize fields. A total of 10 egg batches and 50 nymphs (10 per instar) were used for developmental observations. These were transferred to

plastic containers (20 × 20 × 15 cm) for laboratory rearing. The bottom of each container was lined with blotting paper, and wet cotton balls were placed inside to maintain humidity. Muslin cloth was used to cover the containers for aeration. Throughout the rearing period, all containers were maintained under controlled laboratory conditions of 30 ± 2°C and 75 ± 5% relative humidity. All experiments were conducted with a minimum of five replicates per treatment (n ≥ 5). Data are presented as mean ± Standard Error (SE).

Following oviposition, females were transferred to fresh containers, and egg batches were left undisturbed with wet cotton plugs to maintain humidity. Egg batches were inspected daily to record the incubation period and hatching percentage. Since first-instar nymphs of ***A. spinidens*** are nonfeeders (GholamzadehChitgar *et al*., 2020), neonate nymphs were provided with tender green bean pods along with moist cotton balls (Manley, 1982) to serve as a nutritional supplement during this early stage. Prey was introduced from the second instar onward. Second and third-instar nymphs were offered second-instar larvae of ***S. frugiperda***, while fourth and fifth-instar nymphs and adults were provided with third, fourth, and fifth-instar larvae, thereby matching prey size to the predator's developmental stage and handling capacity (Shintani *et al*., 2010). Fresh prey was replenished twice daily, and larvae were replaced every 24 hours to ensure prey quality. Containers were cleaned regularly, and paper towels were placed inside to absorb waste and provide shelter. All containers were examined at 24hour intervals, with inspections covering the inner walls, the underside of maize leaves used as oviposition substrates, and the muslin cloth covering.

RESULTS

Biology

Eggs

The adult laid 2-6 batches of eggs, with about 10-12 eggs per batch, for a total of about 25-70 eggs in its lifetime. These batches of eggs (Table 1; Figure 1A) were placed on muslin cloth placed over the container. A black-coloured glueing substance on the surface bonded the eggs together upon contact. Within an hour, the newly laid creamy white eggs turned dark brown and golden in colour. The eggs became dark crimson one day before hatching. They were barrel-shaped, ventrally convex, dorsally flattened, and possessed 14-20 micropylar processes. At 30 ± 2°C and 75 ± 5% RH, the hatching rate was 95-100%, and the incubation period ranged from 6 to 7 days, with a mean of 6.33 ± 0.58 days (n = 10 egg batches). Hatching occurred between 7 and 11 a.m.

Nymphs

A. spinidens had a total nymphal period ranging from 17 to 22 days, with a mean duration of 19.33 ± 0.58 days. (n = 50 nymphs). Five moults and five nymphal instars were recorded. Below is a detailed description (Table 1) of the characteristics and duration of each nymphal instar. Compared to later stages, the early nymphal stages exhibited a significantly higher mortality rate.

First Instar: When the cuticle sclerotised, the red colour and black eyes of the newly hatched nymphs changed to black and red, respectively (Figure 1B). The legs, thorax, head, and antennae were all black. The middorsal region of abdominal segments three to seven displayed three black bands on the red abdomen, and the dorsolateral sides of the abdomen had six pairs of black spots. The rostrum and antennae were foursegmented, with the last antennal segment longer than the others. The first nymphal instar lasted 2 to 3 days, with a mean duration of 2.67 ± 0.58 days. First instar nymphs remained on the egg mass for the first two days immediately after hatching.

Second Instar: Apart from an increase in size and the presence of a band on the dorsal surface of the abdomen, the second instar nymph appeared morphologically similar to the first instar (Figure 1C). The abdomen featured four black bands in the middorsal region, spanning the third to seventh abdominal segments. The first and fourth bands were narrower than the two central bands, and the dorsolateral sides of the abdomen each had seven pairs of black spots. The duration of the second instar ranged from 3 to 4 days, with a mean of 3.33 ± 0.58 days.

Third Instar: Third instar nymphs were morphologically similar to the second instar, except for a change in colour from dark brown to black (Figure 1D). Two yellow spots appeared on the second and third abdominal segments, near the wing pad region. As in the second instar, there were seven pairs of black dots on the dorsolateral sides and four black bands on the middorsal region. The third instar lasted 3 to 4 days, with a mean of 3.33 ± 0.58 days.

Fourth Instar: Except for its larger size and the presence of rudimentary wing pads, the fourth instar nymph resembled the third instar (Figure 1E). The duration of the fourth instar was 3 to 4 days, with a mean of 3.67 ± 0.58 days.

Fifth Instar: Compared to earlier instars, the fifth instar nymph was larger. The abdomen turned brown with larger

yellow spots, while the head and thorax appeared black (Figure 1F). Welldeveloped wing pads partially concealed the yellow markings. The fifth instar lasted 6 to 7 days, with a mean of 6.33 ± 0.58 days. This was the longest duration among all instars.

Adult

The recently molted adult bug was orange to pink before turning pale brownish. The mature bug exhibited a pentagonal shape. The long, lobular head featured black lines on either side of the clypeus. The antennae consisted

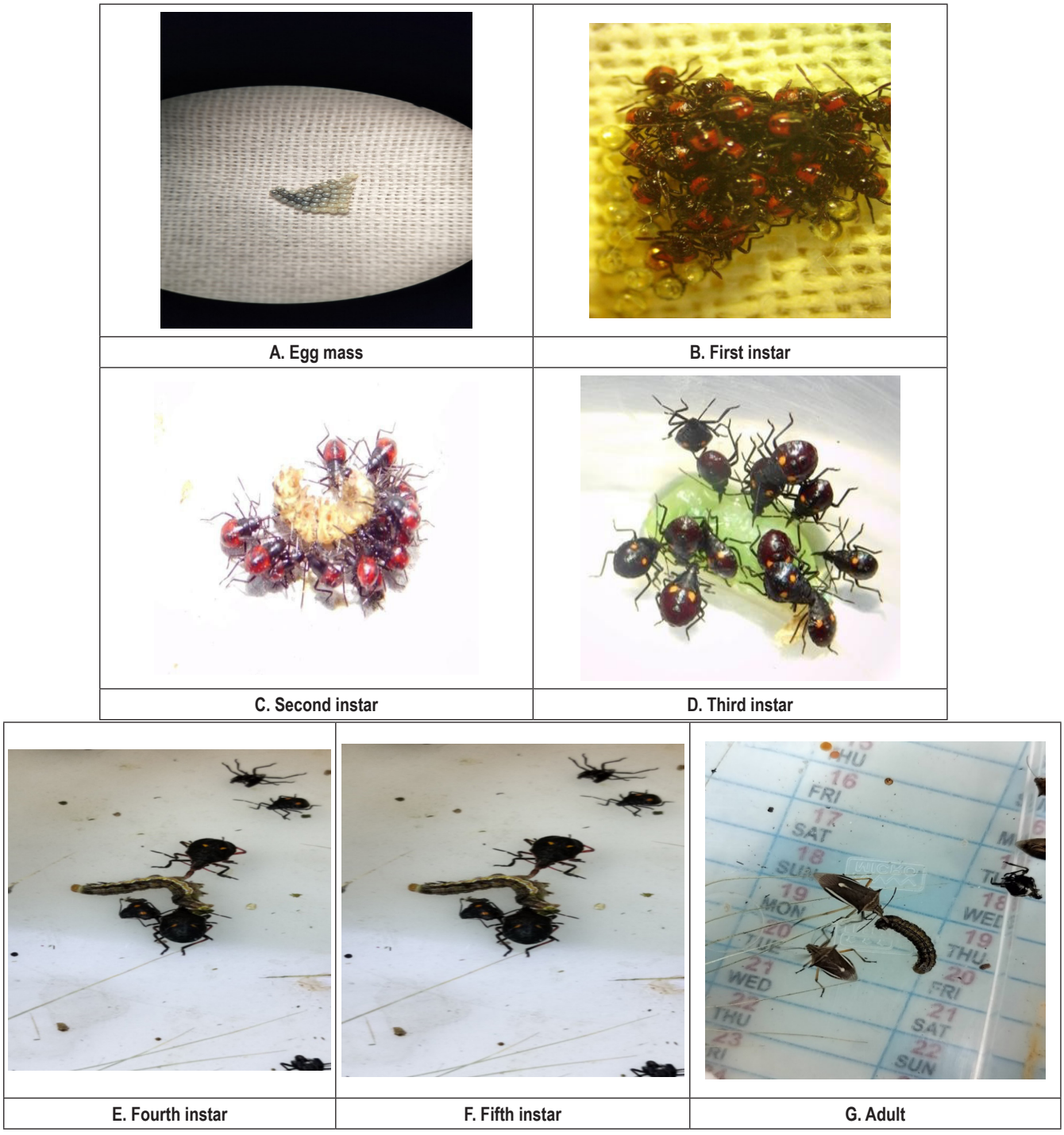


Figure 1. Life stages of *A. spinidens*. **(A).** Egg mass, **(B).** First instar, **(C).** ***Second instar,*** **(D).** ***Third instar,*** **(E).** ***Fourth instar,*** **(F).** Fifth instar, and **(G).** Adult.

Table 1. Developmental biology of *A. spinidens* under laboratory conditions (30 ± 2°C, 75 ± 5% RH; n ≥ 5)

S. No.	Developmental stage	Duration range (days)	Mean ± SE (days)
1	Incubation period	6-7	6.33±0.58
2	Nymph		
	1st instar	2-3	2.67±0.58
	2nd Instar	3-4	3.33±0.58
	3rd Instar	3-4	3.33±0.58
	4th Instar	3-4	3.67±0.58
	5th Instar	6-7	6.33±0.58
3	Total nymphal period	17-22	19.33±0.58
4	Adult	13-16	14.67±1.53
5	Total developmental period (Egg to adult)	36-45	40.33±0.58

of five segments, dark brown and covered with fine setae. The pronotum displayed a lateral pronotal spine, with two yellow bands extending straight from the pronotal angle and a short spine at its base. The scutellum was a long, thin, triangular structure with yellow spots in the middorsal area (Figure 1G). Adult longevity ranged from 13 to 16 days, with a mean of 14.67 ± 1.53 days (n = 10 adults). The total nymphal period was 19.33 ± 0.58 days. The total developmental time from egg to adult ranged from 36 to 45 days, with a mean of 40.33 ± 0.58 days (Table 1).

DISCUSSION

Mass rearing

The successful laboratory rearing of *A. spinidens* is essential for its application as a biocontrol agent. Providing green bean pods for neonates and *S. frugiperda* larvae for later instars significantly influenced growth and survival. A major challenge observed was the predator's sensitivity to temperature and moisture fluctuations, which complicated colony maintenance. Similar issues have been reported in other predatory pentatomids, where variations in environmental conditions negatively affected survival and reproduction (Manley, 1982; Shintani *et al*., 2010). The study also highlighted a dietary shift in *A. spinidens*, with neonates favouring plantbased food while later instars exhibited carnivory, preying on *S. frugiperda* larvae. This indicates that prey availability is critical for sustaining populations under both field and laboratory conditions. Overall, *A. spinidens* can be reared successfully under controlled conditions, but further refinements of rearing protocols are needed to enable largescale production.

Biology

The present findings are consistent with earlier reports while showing some differences attributable to prey type and rearing conditions. The complete life cycle of *A. spinidens* was first documented by Manley (1982), who reported a total developmental period of 32.0 ± 1.0 days (nymphal phase 25.67 ± 0.58 days) on an unspecified prey. Uematsu (2006) recorded 32 days on *S. litura*, while Rao and Rao (1979) reported a shorter maturation period of 24.20 days, reflecting the influence of prey species. Barsagade and Salwe (2020) documented an incubation period of 6.6 ± 0.03 days and instar durations of 2.3, 3.3, 3.6, 3.6, and 6.0 days for first through fifth instars, respectively, with the fifth instar consistently being the longest - a pattern corroborated in the present study (6.33 ± 0.58 days). The black adhesive securing the eggs prevents desiccation, and the rapid colour change indicates embryonic development, as also noted by Manley (1982). The gregarious behaviour of first instars (remaining on the egg mass for two days) likely conserves moisture and reduces predation risk, a trait common among

Asopinae (Shintani *et al*., 2010). The appearance of yellow spots from the third instar onward and the progressive development of wing pads in fourth and fifth instars are consistent with morphological descriptions from other geographical populations (Barsagade & Salwe, 2020). The longest duration of the fifth instar (6.33 days) suggests this stage is critical for accumulating energy reserves needed for adult survival, mobility, and predatory efficiency.

The present study has several limitations. First, it was conducted under controlled laboratory conditions (30 ± 2°C, 75 ± 5% RH), which may not fully represent field conditions where temperature and humidity fluctuate. Second, the sample sizes (10 egg batches, 50 nymphs, 10 adults) meet the nominal requirement of $n \geq 5$, but larger samples could improve precision. Third, only one prey species (*S. frugiperda*) was tested; comparative studies with alternative prey (e.g., *S. litura*, *C. suppressalis*) are needed to assess prey preference and nutritional suitability. Fourth, the study did not evaluate the predator's field performance, its interaction with other natural enemies, or the effect of different host plants on its biology. Finally, the rearing protocol, while effective, remains labourintensive and sensitive to environmental fluctuations, which constrains largescale production.

CONCLUSION

Based on the results and discussion, the following conclusions are drawn:

- *Andrallus spinidens c*ompletes five nymphal instars under controlled laboratory conditions (30 ± 2°C, 75 ± 5% RH), with a mean total developmental period of 40.33 ± 0.58 days from egg to adult.
- The fifth instar is the longest (6.33 ± 0.58 days), while early instars (first to fourth) range from 2.67 to 3.67 days.
- High hatching success (95-100%) and consistent developmental durations confirm that ***A. spinidens*** can be reared successfully on ***S. frugiperda*** larvae.
- Early nymphal instars show higher mortality than later instars, indicating the need for careful humidity and temperature control during laboratory rearing.
- The predator's sensitivity to environmental fluctuations remains a constraint for largescale rearing under current protocols.

FUTURE SCOPE

Massrearing protocols can be refined by testing alternative prey (e.g., factitious prey) or artificial diets. Field studies should evaluate the predation efficiency, functional response, and field persistence of ***A. spinidens*** against fall armyworm in Indian maize agroecosystems to support its deployment in integrated pest management.

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REFERENCES

Barsagade, D. D., and Salwe, A. A. 2020. Studies on biology of pentatomid bug *Andrallus spinide*ns (F) and its predatory potential in rice field of Vidarbha. *Res J Agric Sci*, **11**(6): 1281-1285.

Ebadi, R., and Ghaninia, M. 2003. Study of mass rearing feasibility and egg storage of a predatory bug, *Andrallus spinidens* (F.) (Hemiptera: Pentatomidae) under laboratory conditions. *Iran J Agric Sci*, **34**(1): 137-147.

Gholamzadeh-Chitgar, M., Hajizadeh, J., Ghadamyari, M., Karimi-Malati, A., and Hoda, H. 2020. The stink bug, *Andrallus spinidens* (Hemiptera: Pentatomidae), a potential predator for effective biological control. *J Plant Prot Res*, **60**(4): 336-344. https://doi.org/10.24425/jppr.2020.134754

ICAR-National Bureau of Agricultural Insect Resources. 2020. Biocontrol based IPM management of fall armyworm in maize. https://www.nbair.res.in/node/1449

Khodaverdi, H., Sahagard, A., Amir-Maafi, M., and Mohaghegh, J. 2012. Biology and predatory characteristics of *Andrallus spinidens* (Fabr.) (Heteroptera: Pentatomidae) on larvae of *Spodoptera litura* under field conditions. *J Entomol Soc Iran*, **32**(1): 1-19.

Maheshwari, S., Singh, K. K., Chandana, M. S., Kachave, S. B., Baliyan, S., Mishra, P. K., and Gupta, S. 2023.

Study of ecological diversity of predatory Asopinae bugs in the agroecosystem of the mid-western plains. *Int J Environ Clim Chang*, **13**(10): 3996-4004. https://doi.org/10.9734/ijecc/2023/v13i103075

Manley, G. V. 1982. Biology and life history of the predatory pentatomid *Andrallus spinidens* (Fabricius). *J Georgia Entomol Soc*, **17**(4): 427-432.

Maurya, R. P., Pandey, A. K., and Tiwari, S. 2020. Host range and distribution of predatory stink bug *Andrallus spinidens* (F.) in Uttarakhand. *J Entomol Zool Stud*, **8**(3): 1621-1625.

Mohaghegh, J., and Amir-Maafi, M. 2007. Reproduction of the predatory stinkbug *Andrallus spinidens* (F.) (Heteroptera: Pentatomidae) on live and frozen prey. *Appl Entomol Zool*, 42(1): 15-20. https://doi.org/10.1303/aez.2007.15

Pires, E. M., Nogueira, R. M., Beber, R. C., Junior, W. F., Soares, M. A., Serrão, J. E., and Zanuncio, J. C. 2023. Attack efficacy by *Brontocoris tabidus* and *Podisus nigrispinus* (Hemiptera: Pentatomidae) on *Thyrinteina arnobia* (Lepidoptera: Geometridae) with different pre-release food-free intervals. *Phytoparasitica*, **51**: 967-973. https://doi.org/10.1007/s12600-023-01085-4

Plata-Rueda, A., Martínez, L. C., Zanuncio, J. C., and Serrão, J. E. 2022. Advances zoophytophagous stinkbugs (Pentatomidae) use in agroecosystems: Biology, feeding behavior and biological control. *J Pest Sci*, **95**: 1485-1500. https://doi.org/10.1007/s10340-022-01518-z PMCid:PMC12833490

Rao, P. R. M., and Rao, K. T. 1979. Biology of *Andrallus spinidens* (Fabricius) (Pentatomidae: Hemiptera), a predator of lepidopterous larvae. *Indian J Entomol*, **41**(4): 365-370.

Shanker, C., Sapna, P., Shabbir, K., Sunil, V., Jhansirani, B., and Katti, G. 2017. Biology and functional response of *Andrallus spinidens* (F.) to the rice armyworm *Spodoptera mauritia* (Boisduval). *J Biol Control*, **31**(4): 201-204. https://doi.org/10.18311/jbc/2017/16279

Sharanabasappa, D., Kalleshwaraswamy, C. M., Asokan, R., Mahadeva Swamy, H. M., Maruthi, M. S., Pavithra, H. B., and Goergen, G. 2018. First report of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. *Pest Manag Hort Ecosyst*, **24**(1): 23-29.

Shintani, Y., Masuzawa, Y., Hirose, Y., Miyahara, R., Watanabe, F., and Tajima, J. 2010. Seasonal occurrence and diapause induction of a predatory bug *Andrallus spinidens* (F.) (Heteroptera: Pentatomidae). *Entomol Sci*, **13**(3): 273-279. https://doi.org/10.1111/j.1479-8298.2010.00386.x

Shylesha, A. N., and Sravika, A. 2018. Natural occurrence of predatory bugs, *Eocanthecona furcellata* (Wolff) and *Andrallus spinidens* (Fabr.) on *Spodoptera frugiperda* (Smith) (Hemiptera: Pentatomidae) in maize and their potential in management of fall armyworm. *J Biol Control*, **32**(3): 209-211. https://doi.org/10.18311/jbc/2018/22477

Singh, K. J., and Singh, O. P. 1989. Biology of a pentatomid predator, *Andrallus spinidens* (Fab.) on *Rivula* sp., a pest of soybean in Madhya Pradesh. *J Insect Sci*, **2**: 134-138.

Suby, S. B., Prasanna, B. M., Gupta, R., and Hossain, F. 2020. Invasion of fall armyworm (*Spodoptera frugiperda*) in India and lessons for developing countries. *Curr Sci*, **118**(12): 1910-1914.

Uematsu, H. 2006. Reproductive rate and predatory ability of pentatomid bug *Andrallus spinidens* (Fabr.) (Heteroptera: Pentatomidae). *Jpn J Appl Entomol Zool*, **50**(2): 145-150. https://doi.org/10.1303/jjaez.2006.145

AUTHOR CONTRIBUTION

Srikanth N. conceived and designed the study, conducted laboratory and field experiments, and prepared the initial manuscript draft. S. Saran contributed to methodology development, data analysis, and manuscript editing. Abinaya S. assisted with data compilation and literature review. All authors have read and agreed to the published version of the manuscript.