Indian Journal of Experimental Biology
Vol. 61, January 2023, pp. 66-76
DOI: 10.56042/ijeb.v61i01.69783

Parasitoid complex of legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) on different pulses

S Sambathkumar[1]*, C Durairaj[1], S Mohankumar[2], B Preetha[2], R Aravintharaj[2] & N Ganapathy[1]

[1]Department of Agricultural Entomology; [2]Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore- 641 003, Tamil Nadu, India

Received 24 May 2021; *revised 20 December 2022*

The spotted or bean pod borer, *Maruca vitrata* Fabricius is an important pest of major pulses and vegetable legumes in India and are mostly managed by chemical pesticides. Exploring other alternative management tools, here, we carried out on the availability of parasitoids and its genetic variation to control this major pest. Totally, four larval parasitoids *viz*., *Bassus* sp., *Trathala flavoorbitalis* Cameron, *Phanerotoma hendecasisella* Cameron and an undetermined Braconid wasp were recorded on *M. vitrata* larva. The occurrence of *P. hendecasisella* was reported for the first time from Tamil Nadu, India. The *Bassus* sp. was found to be dominant with the parasitism of 3.0 to 12.7% in different pulses and total parasitism of four parasitoids was maximum in pigeonpea (16.1%). Total parasitism had a positive relationship with number of webbings on cowpea. The larval parasitoids *Bassus* sp. and braconid wasp (undetermined) yielded specific fragments (~800 bp) with mitochondrial COI primer. Presence of *Wolbachia* was confirmed in all four larval parasitoids with the amplicons size between 600 and 650 bp. The results clearly indicate the close proximity of *Bassus* sp. on *M. vitrata* than other parasitoids studied, and suggestfor further insights on suitability, mass culturing and development for sustainable management of this insect pest.

Keywords: Bean pod borer, Cytochrome c oxidase I, Larval parasitoids, Spotted pod borer, *Wolbachia*

Insect pod borer complex play a vital role in affecting the yield of majority pulses. Among them, the spotted or bean pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) is an important pest of grain legumes[1] and major leguminous vegetables[2] in India. The larvae cause damage to the flower buds, flowers and pods through their webbing and additionally contaminate with their excreta and frass[3]. Due to lack of effective alternative control measures, farmers mainly rely on use of various insecticides against *M. vitrata*. Moreover, on pulses like greengram, blackgram, cowpea and lablab, use of insecticides are less appreciated due to their short post-flowering period and their rainfed method of cultivation. However, farmers spray different insecticides at higher dosage with more frequencies to achieve effective control and mostly resulted in lesser penetration of sprayed chemicals on the target feeding sites of these crops[4]. During many instances, failure of insecticides is encountered mainly due to the development of resistance in *M. vitrata*[5,6] and also it poses various ill effects in the environment. The concealed feeding of this pest also complicates its control by pesticides due to hindrance in penetration. Hence, there is an increased demand for some other effective management tactics along with the present practices that are sustainable and eco-friendly.

The occurrence of natural enemies particularly hymenopterous parasitoids on *M. vitrata* has been studied scarcely on various host plants. This forms a major gap in our understanding on ecology of this pest, with significant implications for its management on other host plants also. Tritrophic interactions involving the plants and herbivores are of great importance in shaping population dynamics of *M. vitrata* and their natural enemies[7]. Biocontrol agents especially, the parasitoids play a crucial role in IPM of many insect pests and have good compatibility with other IPM methods. In these contexts, here, we explored the available parasitoids and their potential against *M. vitrata.*

Material and Methods

Collection and identification of parasitoids

Larvae of *M. vitrata* infested flowers and pods were collected from the crop fields (11^{O} 02’32” N

*Correspondence:
E-Mail: sambathagritech@gmail.com

latitude, 76^{O} 92'94" E longitude and 426.72 m altitude) of pigeon pea (cv. CO-RG-7), green gram (cv. CO-GG-7), black gram (cv. CO-BG-6), cowpea (cv. CO-7) and lablab (cv. Rohini) at Research Farm, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India at monthly intervals. Infested flowers and pods were immediately brought to laboratory and 50 larvae of various stages were randomly selected and reared separately on the host from which they were collected until their pupation. Similarly, three replications were maintained on each host crop. Culturing was done in plastic troughs (30 cm diameter) with a layer of clean filter paper at bottom and was regularly examined for the presence of any parasitoids from the larvae and pupae. The emergence of the larval stages of parasitoids, their pupation and adult emergence were recorded and per cent parasitism was calculated based on the number of parasitoids emerged out of totally released 50 larvae. The emerged parasitoids were identified by Indian Council of Agricultural Research- National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bengaluru, Karnataka, India.

Effect of weather factors on the per cent parasitism

The weather data on maximum temperature (°C), minimum temperature (°C), maximum relative humidity (%), minimum relative humidity (%), rainfall (mm), sunshine (h.), wind velocity (kmph) and evaporation (mm) were obtained from the Agro Climate Research Centre (ACRC), TNAU, Coimbatore, Tamil Nadu, India for the entire study period and their fortnight average was calculated. The individual and total per cent parasitism by all parasitoids were recorded at monthly intervals and correlated with the weather parameters using the percent parasitism as dependent variable (Y) and each of the weather parameters as independent variable (X). The mean weather parameters that prevailed during the previous 30 days of observation day and their mean values were taken into account for this study. Correlation and multiple regression analysis were performed with weather parameters using SPSS 16.0 statistical software[8].

Correlation co-efficient (r), was computed by adopting the standard statistical formula by Karl Pearson[9].

$$r = \frac{\sum XY - (\sum X) \times (\sum Y)/n}{[\sum X^2 - (\sum X^2/n)/\sum Y^2 -](\sum Y^2/n)^{1/2}}$$

The correlation co-efficient (r) was tested for significance or non significance by Fisher 't' test and is defined as

$$r = \frac{r}{(1-r2)^{1/2}} \times (n-2)^{1/2}$$

with (n-2) degrees of freedom. Multiple regression analysis was also performed with weather parameters. A measure of goodness of fit the values of Coefficient of determination (R^2)[10] was calculated to develop simple models as below.

$$(R^2) = 1-[\sum (Y_i - Y\hat{})^2 / [\sum (Y_i - Y-)^2].$$

where, Y_i represents the parasitoid abundance on *M. vitrata* at time 't'.

DNA extraction

DNA was extracted from individual parasitoids using modified CTAB method[11]. Briefly, single larva of *M. vitrata* was separately taken in an eppendorf tube and 500 µL CTAB buffer was added. Samples were crushed with micro pestle and were kept for incubation at 65°C for 1 to 2 h on water bath with intermittent stirring. After incubation, the tubes were allowed to cool at room temperature. The samples were centrifuged at 12000 rpm for 10 min at 4°C. The aqueous phase was transferred to another micro centrifuge tube without disturbing the inner phase and 300 µL of ice cold mixture of chloroform and isoamyl alcohol (24:1) were added to another new tube, and it was again centrifuged at 12000 rpm for 15 min at 4°C. The top layer (contain DNA) in the tube was alone collected and transferred to another micro centrifuge tube without disturbing subsequent layers. Again, 350 µL of ice cold isopropanol was added in to new tube and tube was incubated at –20°C for overnight. Then samples were centrifuged at 12000 rpm for 10 to 30 min at 4^{o} C depend on the pellet. About 200 µL of 70% ice cold ethanol was added in to the tubes containing pellets and again centrifuged at 12000 rpm for 10 to 15 min at 4^{o} C to remove impurities. The pellet settled at the bottom of the tube was air dried completely (30 to 40 min) after discarding ethanol. Finally, DNA was dissolved in 30 µL of TE buffer. The DNA was digested with proteinase K and RNase at 37°C for 30 min to remove protein and RNA contaminations. The extracted DNA was stored at –20°C and used in further analyses.

Characterization of *M. vitrata* parasitoids using COI primers

For the Mitochondrial DNA variation with respect to the parasitoids, the samples were subjected to PCR amplification used a COI primer sets *viz*., C1-J 2183 (TTGATTTTTTGGTCATCCAGAAGT) and L2-N-3014 (TCCAATGCACTAATCTG CCATATT) which amplified the mitochondrial region of Cytochrome oxidase subunit I (COI).

Polymerase chain reactions were performed in 25 µL volumes in a thermocycler (Master Cycler, Eppendorf, Germany). The composition of cock tail mixture (for 23 µL reaction mix) is as follows. Totally 25 µL volume contained 2 µL template DNA, 16.0 µL of sterile water, 2.5 µL 10×PCR buffer (1.2 µM) 1.0 µL of $MgCl_2$ (1.2 µM), 1 µL dNTP mixtures (200µM of each of dATP, dCTP, dGTP, and dTTP), 1 µL of both forward and reverse RAPD primer (10 µM), and 0.5 µL Taq DNA polymerase (1.5 Units). PCR (thermocyclic conditions) were optimized to achieve informative and reproducible fingerprinting profiles using 2.5 µL of template DNA, and amplification was achieved by the following programme: 94°C for 2 min (initial denaturation), 94°C for 1 min, 55°C for 1 min (annealing) and 72°C for 1 min (extension), This cycle was repeated 40 times, which was followed by a final extension of 72°C for 2 min.

Finally 5 µL of amplified PCR products were separated by agarose (1.5%) gel electrophoresis in TBE buffer at 70 V for 45 min and visualized in ethidium bromide stained gel on UV transilluminator and documented in Image documentation system (Bio-Rad, USA). The size of individual DNA fragments was compared with a co-migrating 100 bp DNA ladder (MBI Fermentas, Vilnius, Lithuania).

Sequencing of PCR product

The amplified PCR fragment products of *Maruca* parasitoids were sequenced at Sci Genome Labs Pvt. Ltd. Kerala, India. The successful nucleotide sequences were compared with already available reference sequences of *M. vitrata* parasitoids available at National Centre for BioInformatics (NCBI) GenBank and subjected to phylogenetic analysis by Basic Local Alignment Search Tool (BLAST) using BioEdit version 7.0.9.0[12]. This was done to identify the similarity among parasitoids.

Studies on characterization of *M. vitrata* parasitoids using *Wolbachia* specific primers

DNA was individually extracted[11] from four parasitoids of field collected larval populations as mentioned previously and stored template DNA amplified with the *Wolbachia* primer sets *viz*., wsp81f (TGGTCCAATAAGTGATGAAGAAAC) and wsp691r (AAAAATTAAACGCTACTCCA)[13] and ftsZAdf (CTCAAGCACTAGAAAAGTCG) and ftsZAdr (TTAGCTCCTTCGCTTACCTG)[14].

PCR reactions were performed in 25 µL reaction volumes that contained 2µL template DNA, 16.0 µL of sterile water, 10 x of 2.5 µL of Taq buffer of 1.2 µM, 1.0 µL of $MgCl_2$ (1.2 µM), 1 µL of 200 µM of each dNTPs, 1 µL each of forward and reverse primer of 10 µM concentración along with 0.5 µL of taq polymerase (1.5 units). Thermal cycling was carried out on an Eppendorf thermo cycler and performed using a 94°C denaturation step for 5 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, followed by final extension cycle of 72°C for 7 min. A 5 µL aliquot of PCR product was separated on a 1% agarose gel that contained 0.5 µg/mL ethidium bromide at 70 V for 45 min and viewed under UV light and documented in image documentation system (Bio-Rad, USA).

Results

Larval parasitoids

Studies on the parasitoid complex of *M. vitrata* revealed the occurrence of four hymenopteran parasitoids *viz*., *Bassus* sp., (Braconidae) *Trathala flavoorbitalis* Cameron (Ichneumonidae), *Phanerotoma hendecasisella* Cameron (Braconidae) and an undetermined Braconid wasp (Fig. 1(a-d) & 2(a-d)). Of which, the occurrence of *P. hendecasisella* on *M. vitrata* is first time reported from Tamil Nadu, India. The keys for the parasitoid identification are described below.

***Bassus* sp. (Braconidae:Agathidinae)**

Diagnosis: Pronotum, mesoscutum, scutellum, mesopleuron and ovipositor red brown; apical half of fore femur, fore tibia, fore tarsus, mid tibia, mid tarsus and mid tibial spurs yellowish. Head, antennae, propodeum, basal half of fore femur, mid femur almost entirely, hind legs and metasoma black. Mesoscutum

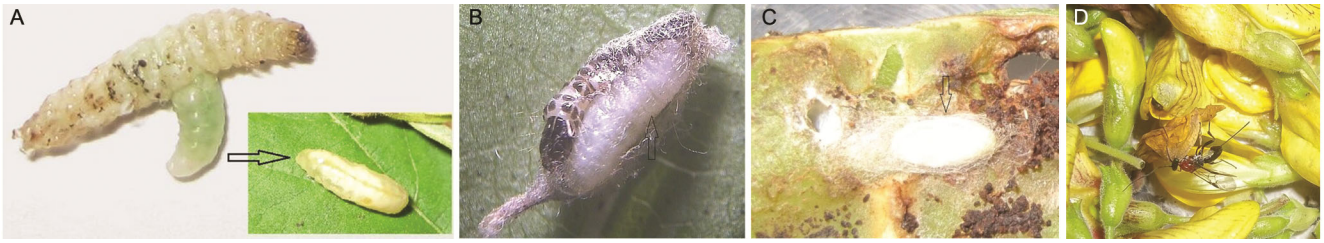

Fig. 1(A-D) — (A) Grub of larval parasitoid, *Bassus* sp; (B) Cocoon of *Bassus* sp; (C) Pupa of *Bassus* sp; and (D) Female wasp of *Bassus* sp.

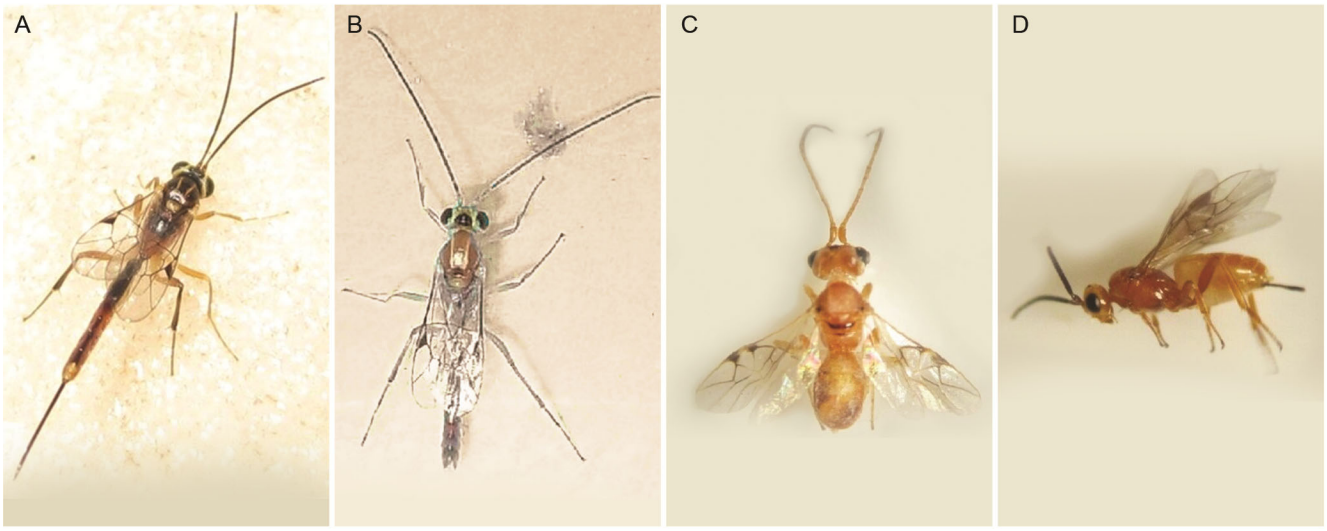

Fig. 2(A-D) — (A) Female wasp of larval parasitoid, *Trathala flavoorbitalis*, (B) Male wasp of larval parasitoid, *Trathala flavoorbitalis*, (C) *Phanerotoma hendecasisella* and (D) Braconid wasp

sparsely punctuate; notauli distinct. Metasoma smooth and shiny; first tergite with carina; second tergite smooth and shiny. Ovipositor moderately long.

***Phanerotoma hendecasisella* (Braconidae:Cheloninae)**

Diagnosis: Body colour pale testaceous. Antennae yellowish brown, infuscated at apex; wings hyaline; pterostigma fuscous, paler at the base and apex. Mesosoma fuscous; notauli faintly indicated basally. Scutellum apex with a broad shining black transverse strip. Metanotum with black infuscation medially at posterior margin. Propodeum yellow brown. Hind femur with apical half yellow brown and basal half testaceous; hind tibia medially with a white-pallid band, apical half brown. Basal two segments of metasoma medially pale testaceous; rest metasoma brown.

***Trathala flavoorbitalis* (Ichneumonidae:Cremastinae)**

Diagnosis: Yellow orange species; first and second tergites black brown while the remainder of the metasoma yellow orange. Head yellow, antenna brown, scape and pedicellus yellowish ventrally. Mesonotum yellow orange, dorsal brown marking on mesoscutal lobes, around scutellum. Notauli yellow. Tegulae and scutellum yellow. Metasoma yellow orange, first and second tergites and basal triangle on third tergite dark brown, rest tergites brown dorsally and yellow orange laterally. Legs yellow, hind tibia slightly infuscate basally and apically; wings hyaline, pterostigma brown, its anterior half yellow. Antenna with 30-33 flagellomeres. Mesonotum and mesopleuron densely punctuate, scutellum and post scutellum more sparsely punctate. Propodeum more densely punctate-shagreened dorsally than laterally. First tergite a little longer than second.

Table 1 — Species complex of larval parasitoids (TF, *Trathala flavoorbitalis* and PH, *Phanerotoma hendecasisella*) of *M. vitrata* and their levels of parasitism

Month	Parasitism* (%)				
	Bassus sp.	TF	PH	Braconid wasp	Total
January	2.0^{b} (8.1)	0.0^{b} (0.0)	0.0^{c} (0.0)	0.0^{c} (0.0)	2.0
February	2.0^{b} (8.1)	1.0^{b} (5.7)	0.0^{c} (0.0)	0.0^{c} (0.0)	3.0
March	3.0^{b} (10.0)	0.0^{b} (0.0)	0.0^{c} (0.0)	0.0^{c} (0.0)	3.0
April	3.3^{b} (10.5)	0.0^{b} (0.0)	0.0^{c} (0.0)	0.0^{c} (0.0)	3.3
May	10.0^{ab} (18.4)	6.0^{a} (14.2)	1.0^{bc} (5.7)	0.0^{c} (0.0)	17.0
June	4.0^{ab} (11.5)	5.0^{a} (12.9)	0.0^{c} (0.0)	0.0^{c} (0.0)	9.0
July	4.0^{ab} (11.5)	5.3^{a} (13.4)	2.0^{bc} (8.1)	0.0^{c} (0.0)	11.3
August	4.0^{ab} (11.5)	1.3^{b} (6.6)	1.7^{bc} (7.4)	0.0^{c} (0.0)	7.0
September	9.5^{ab} 18.0)	3.5^{a} (10.8)	7.5^{a} (15.9)	0.0^{c} (0.0)	20.5
October	4.0^{ab} (11.5)	4.2^{a} (11.8)	4.2^{b} (11.8)	2.0^{a} (0.0)	14.4
November	9.3^{ab} (17.8)	4.0^{a} (11.5)	0.0^{c} (0.0)	0.7^{b} (4.7)	14.0
December	12.7^{a} (20.8)	0.1^{c} (1.5)	2.1^{bc} (8.3)	0.4^{bc} (3.6)	15.3
SEd	4.6028	1.8383	1.4539	0.3231	-
CD P=0.05)	9.2204	3.6825	2.9124	0.6472	-

[*Mean levels of parasitism by individual species collected form 5 hosts in a month, figures in the parentheses are arcsine transformed values. In a column mean(s) followed by a common letter are not significantly different at 5 % in DMRT]

Abundance of parasitoids on different pulses

***Bassus* sp.**

The results revealed that the parasitism of *Bassus* sp. on *M. vitrata* was significant and maximum with larvae collected from pigeon pea (12.7%) followed by cowpea (7.8%) and lablab bean (6.2%), respectively (Table 1). Whereas, on green

gram and black gram, it was 4.3 and 3.0%, respectively. The highest parasitoid adult emergence was recorded in lablab bean (75%) followed by cowpea (58%) and pigeon pea (51.4%) as against no emergence on black gram. The pupal mortality of *Bassus* sp. ranged from 15.6 to 32.1% on green gram and pigeon pea, respectively. The complete pupal mortality of *Bassus* sp. was recorded on black gram.

Trathala flavoorbitalis

The parasitism was found to be low in all crops and a significant maximum of 4% was recorded on *Maruca* larvae collected from lablab bean as compared pigeon pea (1%) and black gram (1%) (Table 1). Adult emergence of *T. flavoorbitalis* was found to be maximum on green gram (50%) as against black gram (0.0%). The pupal mortality of *T. flavoorbitalis* was minimum on *Maruca* larvae collected from green gram (0.0%) followed by 8.9 and 10.7% on cowpea and pigeon pea, respectively when compared to complete mortality (100%) on black gram.

Phanerotoma hendecasisella

There was no significant variation with respect to parasitism *by P. hendecasisella* on *M. vitrata* in different pulses which ranged from 1.0 to 2.0% (Table 1). Similarly, no variation was registered on adult emergence and pupal mortality of *P. hendecasisella* from the parasitized larvae. The per cent adult emergence ranged from 0.0 to 16.7% on black gram and green gram. There was no pupal mortality on green gram and black gram populations as against 16.4% on *Maruca* larvae collected from pigeon pea.

Braconid wasp

The results on parasitism by the braconid wasp, its adult emergence and pupal mortality showed an insignificant difference among different hosts. The per cent parasitism ranged from 0.0 in black gram and lablab bean to 0.7 in green gram populations of *M. vitrata.* The adult emergence was recorded only from cowpea (8.3%) and green gram (16.7%) and the pupal mortality of 14.3% was recorded from *M. vitrata* populations of pigeon pea (Table 1).

Occurrence of parasitoid complex of *M. vitrata* on different pulses

The results revealed that the parasitism of *Bassus* sp. on *M. vitrata* larvae was significant and maximum (12.7%) in pigeon pea followed by cowpea (7.8%) and lablab bean (6.2%) when compared to 4.3% in green gram and 3.0% in black gram (Fig. 3). The parasitism of *T. flavoorbitalis* was found to be low (1-4%) in all pulses studied and similar results were also obtained for *P. hendecasisella* (1.0 to 2.1 %). The abundance of braconids wasp was registered only during October (2.0%) to December (0.4%).

The results on total parasitism by different parasitoid species on *M. vitrata* showed a maximum parasitism (16.1%) on larvae collected from pigeon pea followed by lablab bean (11.9%) and cowpea (11%). On green gram and black gram, the parasitism was only 6.9 and 6.1%, respectively.

Correlation and regression between total percent parasitism and incidence of *M. vitrata*

Results on correlation analysis (Table 2) revealed that positive relationship exerted between total percent parasitism and number of webbings on cowpea (r = 0.847*). The multiple regression analysis showed a R^2 value of 0.869, which indicated that 86.9% of variation in total parasitism, was influenced on the *Maruca* larval population in pigeon pea, cowpea and lablab bean together.

$$\text{Total per cent parasitism (Y)} = 2.329 + 0.195X_1 + 0.582^*X_2 - 0.470X_3$$

The equation clearly indicated that in cowpea, one number increase in *Maruca* webbing is expected to

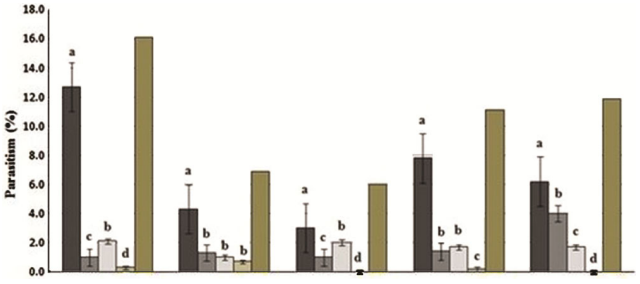

Fig. 3 — Larval parasitism of *Maruca vitrata* in different pulses: One-way ANOVA, $P < 0.05$,$n = 50$ per replication and three replications per host crop, the error bars indicate the standard error, Different alphabets on the error bar for the host crop are statistically significant at $P < 0.05$.

Table 2 — Correlation and regression analysis between total parasitism and incidence of *Maruca vitrata* and different pulses (n = 14)

Variable	Corr. coeff.	Beta wt.	Regr. coeff.	SE	't' val.	't' probability
a (intercept)	1.000	-	2.329^{**}	0.579	4.021	0.028
Pigeon pea	0.394^{NS}	0.268	0.195^{NS}	0.156	1.245	0.301
Cowpea	0.847*	0.715	0.582*	0.174	3.234	0.048
Lablab	-0.483^{NS}	–0.327	-0.470^{NS}	0.311	–1.509	0.228

[R^2 value = 0.869, 'F' value = 6.619, *Significant at 5%, **Significant at 1%, ^{NS} Non significant. SE, Standard error]

increase the total parasitism by 58.2%. This showed the density of host larvae had more influence on total parasitism.

Influence of weather parameters on the population of *Bassus* sp.

Among four larval parasitoid species recorded, the occurrence of *Bassus* sp. was predominant and hence, the correlation and regression analysis was made only to this parasitoid (Table 3). The parasitism by *Bassus* sp. had significant negative correlation with minimum temperature (r = -0.591*) and evaporation (r = -0.799**), whereas positive correlation was obtained with maximum RH (r = 0.642). The results of multiple regression analysis showed a R^2 value of 0.896 revealing that 89.6% of the variation in the parasitism by *Bassus* sp. was influenced by weather parameters (Table 4). The following equation was derived from the regression analysis.

Per cent parasitism by *Bassus* sp. (Y) = $18.438^{**} - 0.644^{**}X_1 - 2.607^{**}X_2 + 2.053^{**}X_3 - 1.447^{**}X_4 - 0.818^{**}X_5 - 9.227^{**}X_6 + 0.959^{**}X_7 + 5.505^{**}X_8$

Table 3 — Correlation matrix of the relationship between total parasitism on *Maruca vitrata* and weather parameters (n = 12)

Parameter	Correlation coefficient	
	Bassus sp.	Total parasitism
Y – Per cent parasitism (%)	1.000	1.000
X_1- Max. temp. (°C)	-0.555^{NS}	–0.576*
X_2 - Min. temp. (°C)	–0.591*	-0.348^{NS}
X_3 - Maximum RH (%)	0.642*	0.593*
X_4 - Minimum RH (%)	0.185^{NS}	0.470^{NS}
X_5 - Wind speed (kmph)	-0.529^{NS}	-0.352^{NS}
X_6 - Sunshine hrs	-0.058^{NS}	-0.267^{NS}
X_7 - Rainfall (mm)	0.189^{NS}	0.376^{NS}
X_8 - Evaporation (mm)	–0.799**	–0.635*

[*Significant at 5%, **Significant at 1%, ^{NS} – Non significant]

Table 4 — Multiple regression analysis of per cent parasitism of *Bassus* sp. on *Maruca vitrata* and weather parameters (n = 12)

Variable	Beta wt.	Regr. Coeff.	SE	't' value	't' probability
a (Intercept)	-	18.438**	64.248	0.287	0.793
X_1 (Max. temp.)	–0.240	–0.644*	2.182	–0.295	0.787
X_2 (Min. temp.)	–1.032	–2.607**	1.828	–1.426	0.249
X_3 (Max. RH)	1.511	2.053**	0.863	2.380	0.098
X_4 (Min. RH)	–1.928	–1.447**	0.591	–2.450	0.092
X_5 (Wind speed)	–0.425	-0.818^{NS}	1.204	–0.680	0.545
X_6 (Sunshine h)	–2.239	–9.227**	1.523	–6.057	0.009
X_7 (Rain fall)	0.665	0.959**	0.355	2.702	0.074
X_8 (Evaporation)	1.077	5.505**	2.590	2.126	0.124

[R^2 value = 0.896, 'F' value = 15.487, *Significant at 5%, **Significant at 1%, ^{NS} – Non significant]

The equation indicated that, for every one degree decrease in maximum and minimum temperature, one per cent decrease in minimum RH and one kmph decrease in wind speed, the parasitism increased by 0.644, 2.607, 1.447, 0.818 and 9.227 units, respectively. Whereas, one per cent increase in maximum RH, one hour increase in sunshine, one mm increase in rainfall and one mm increase in evaporation is expected to raise the parasitism by 2.053, 0.959 and 5.505 unit, respectively.

Influence of weather parameters on total parasitism on *M. vitrata*

The total parasitism by four parasitoids on *Maruca vitrata* larvae had negative relationship with mean maximum temperature (r = -0.576*), mean evaporation (r = -0.635*) and exhibited positive correlation with mean maximum RH (r = -0.593*). Other factors did not show any significant impact on per cent parasitism on *M. vitrata* (Table 3). Results of the multiple regression analysis showed a R^2 value of 0.976 (Table 5) revealing that 97.6% variation in overall parasitism of *M. vitrata* parasitoids was influenced by weather parameters.

Total per cent parasitism (Y) = $-57.232^{**} - 7.473^{**}X_1 + 2.659^{**}X_2 + 2.206^{**}X_3 - 0.931^{**}X_4 - 0.659^{*}X_5 + 2.749^{**}X_6 + 1.653^{**}X_7 + 16.598^{**}X_8$

The result indicated that an increase in one degree of minimum temperature, one per cent of maximum RH, one mm of rainfall and evaporation would lead to an increase of 4.24, 2.94, 0.95 and 7.85 times, respectively. On the other hand, a decrease of one degree maximum temperature, one per cent minimum RH and one kmph of wind speed is expected to increase the parasitism on *M. vitrata* by 7.47, 0.93 and 0.66, respectively.

Table 5 — Multiple regression analysis of total parasitism of parasitoic complex on *Maruca vitrata* and weather parameters (n = 12)

Variable	Beta wt.	Regression coefficient	Standard error	't' value	't' probability
a (Intercept)	-	–57.232**	108.986	–0.525	0.636
X_1 (Max. temp.)	–2.167	–7.473**	2.878	–2.596	0.081
X_2 (Min. temp.)	0.834	2.659**	2.943	0.903	0.433
X_3 (Max. RH)	1.258	2.206**	1.174	1.880	0.157
X_4 (Min. RH)	–1.043	-0.931^{NS}	0.583	–1.597	0.209
X_5 (Wind speed)	–0.277	-0.659^{NS}	2.044	–0.322	0.768
X_6 (Sunshine h)	0.551	2.749**	2.500	1.100	0.352
X_7 (Rain fall)	0.959	1.653**	0.477	3.465	0.040
X_8 (Evaporation)	2.301	16.598**	3.655	4.542	0.020

[R^2 value = 0.976, 'F' value = 7.562, **Significant at 1%, ^{NS} – Non significant. RH Relative humidity]

Genetic variation in *M. vitrata* parasitoids using COI primers

Genetic variations based on the cytochrome c oxidase subunit 1 (COI) primer profile of *M. vitrata* parasitoids (Fig. 4) revealed amplicons of ~800 bp in two parasitoids of *M. vitrata viz.*, *Bassus* sp. and a braconid wasp (Table 6).

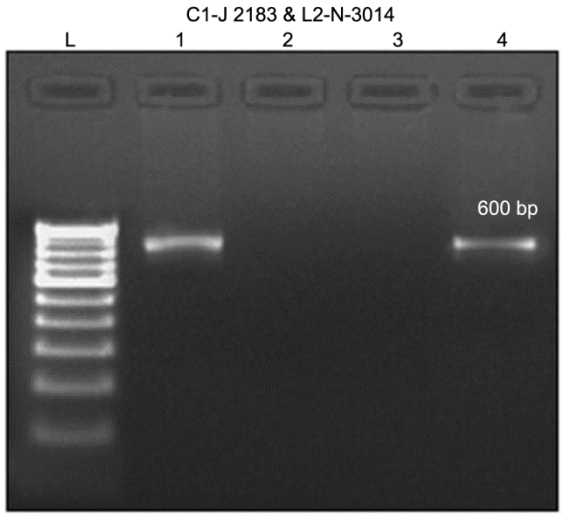

Fig. 4 — COI primer profile of parasitoids of *M. vitrata* (C1-J 2183 & L2-N-3014). Lane L- 100 bp ladder. Lane 1 - *Bassus* sp., Lane 2 - *Trathala flavoorbitalis,* Lane 3 - *Phanerotoma hendecasisella* and Lane 4 – An undetermined braconid wasp

Among four parasitoids recorded on *M. vitrata* larvae, *Bassus* sp. alone yielded successful sequence and it was deposited in GenBank. The resulted partial sequences of COI gene amplified *Bassus* sp. (Fig. 5) confirmed to the maximum similarity with *Therophilus* sp. (93%) and *Agathis* sp. (89%).

Detection of *Wolbachia* endosymbiont in the larval parasitoids of *M. vitrata*

The amplified PCR product indicated the presence of *Wolbachia* in all four parasitoids of *M. vitrata* larvae (Table 6) whereas, the primer ftsZ Adf and ftsZ Adr did not produced any alleles with the parasitoids. The PCR amplified product of *wsp* gene specific primer (wsp81f and wsp691r) indicated presence of few variations at nucleotide

Table 6 — COI primer reaction and *Wolbachia* infection status in different parasitoids of *Maruca vitrata* larvae

Parasitoid	*C1-J-2183* and *L2-N-3014*	*wsp81f* and *wsp691r*	*ftsZ Adf* and *ftsZ Adr*
Bassus sp.	+	+	-
Trathala flavoorbitalis	-	+	-
Phanerotoma hendecasisella	-	+	-
Braconid wasp	+	+	-

[‘+’ amplified; ‘-’ not amplified]

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                    10        20        30        40        50        60        70        80        90       100
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Bassus sp. CCCGCCTTATCTGCGCATTATTATTTTAAATAAACTATGTTTCTTTTATTATTACTATTGATTTAAGGAGAAAGAACTTTTGGTTTATTGGGAATAATTT

                   110       120       130       140       150       160       170       180       190       200
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Bassus sp. ATGCTATATTAACTATTGGATTTTTAGGGTTTATTGTTTGAGCTCATCATATATTTACTATTGGAATAGATGTTGATACACGAGCTTATTTTACATCTGC

                   210       220       230       240       250       260       270       280       290       300
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Bassus sp. ACTATAATTATTGCTATTCCTCTGGTATTAAAATTTTTAGTTGATTAAGAACTTATAATGGAGTAAAAATAAAAATATCTTTAAATATAATATGAAGAAT

                   310       320       330       340       350       360       370       380       390       400
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Bassus sp. AGGATTTATTTTTTTATTTACATTAGGAGGAGTTCTGGAGTTATTTTATCTAATTCTTCTGTTGATATTATTTTACATGATACTTATTATGTTGTAGCAC

                   410       420       430       440       450       460       470       480       490       500
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Bassus sp. ATTTTCATTATGTTTTATCAATAGGAGCTGTATTTGCAATTTTTTCTGGATTTATTTTTTGATTTAATTTATTTTTTGGTTTAAGTTTAAATGAAAATTG

                   510       520       530       540       550       560       570       580       590       600
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Bassus sp. AATAAAAATTCAATTTATAATTATATTTTTTAGGAGTAAATTTAACATTTTTTCCTCAACATTTTTTAGGATTAGGAGGAATACCTCGTCGATATAGAGAT

                   610       620       630       640       650       660       670       680       690       700
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Bassus sp. TATCCTGATATATATATATCTTGAAATATTATTTCATCTTTAGGTTCTATAATACTTTAATTTCTATTTTTATATTAATTTTTTGTTTATTAGAATCTTT

                   710       720       730       740       750       760       770       780       790       800
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Bassus sp. TATTTCAATTCGATTAAAAATTTTTAATAAATATATAAATTCTTCAATTGAATTTTTACAAAATTATCCACCTCAAATTCATAGTATATCAATAAACTTA

                   810       820       830
                    ....|....|....|....|....|.
Bassus sp. GTTATTTAAAATATAAGGGCGCGGCCGCGGC
```

Fig. 5 — Alignment of ~800 bp partial sequence of *Bassus* sp. with COI primer (C1-J-2183 & L2-N-3014)

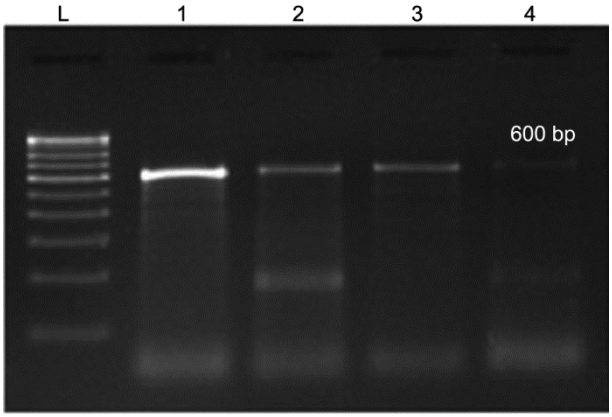

Fig. 6 — *Wolbachia* specific primer profiles of parasitoids of *Maruca vitrata* (wsp81f and wsp691r). Lane L- 100 bp ladder, Lane 1 - *Bassus* sp., Lane 2 - *Trathala flavoorbitalis*, Lane 3 - *Phanerotoma hendecasisella* and Lane 4 – An undetermined braconid wasp

level in four parasitoids of *M. vitrata* with the amplicon size of 600 bp (Fig. 6).

Discussion

The research works on natural enemies especially the parasitoids of *M. vitrata* in India are very limited and only the parasitism by *Phanerotoma* sp. and *Bracon greeni* F.[15] and *B. hebetor* Say had been reported[16-18]. The present findings of parasitoids, such as *P. hendecasisella* (first time from Tamil Nadu) and *Bassus* sp. will be helpful for further research on its exploitation in the biocontrol program of *M. vitrata*. But, many records on *Maruca* parasitoids have been reported from foreign countries. In Nigeria, *P. leucobasis* Kriechbaumer registered as an ovolarval parasitoid on second instar larvae of *M. vitrata*[19]. In Taiwan, the predominant parasitism by *Apanteles taragmae* Viereck[20,21] and *B. asper* Chou & Sharkey[20] along with other hymenopterous parasitoids like *Dolichogenidia* sp., *Trichomma* sp., *Triclistus* sp. and *Plectochorus* sp.[20] were recodred on *M. vitrata* larvae infested on *Sesbania cannabina* Poir. It was also found that *A. taragamae*, was able to mediate the transfer of *M. vitrata* multi-nucleopolyhedrosis virus (*MaviMNPV*) among larval populations of *M. vitrata*[21].

Similar to the present findings, the parasitism of *Therophilus javanus* Bhat and Gupta[22], *T. flavoorbitalis*[22], *T. marucae* van Achterberg and Long[22-24] and *Phanerotoma philippinensis*[23] and *P. syleptae*[25] had been reported on II to IV instar stages of *M. vitrata*. Later in Karnataka, occurrence of parasitoids such as *B. relativus* (Bhat & Gupta) *P. hendecasisella* and *T. flavoorbitalis* were recorded[25]. Hence, occurrence of these parasitoids in the present study on pigeon pea, green gram, black gram and lablab bean is possible in Tamil Nadu. The present findings are slightly deviating from the observations of Huang *et al*. (2003)[20] in Taiwan who recorded maximum parasitism by different larval and pupal parasitoids during June to August months and the variation might be attributed to the difference in the weather parameters prevailed in the field conditions. According to Don-Pedro (1983)[26] *Phanerotoma* sp. and *Braunsia sp.* were the most important larval parasitoids in Nigeria on *M. vitrata.* Interestingly, the preference of these parasitic wasps is highly decided by host induced plant volatiles (HIPV)[27] that are emanated up on infestation by the insect pest. Attraction of *A. taragamae*[28] and *T. javanus*[29] in response to odour of *M. vitrata* damaged cowpea flowers and pods are already reported. Hence, it is clear that HIPVs are also playing crucial role in parasitism on *M. vitrata*.

Positive relationship of total percent parasitism with number of *Maruca* webbings on cowpea (r = 0.847*) revealed there could be considerable levels of both functional and numerical response of larval parasitoids on cowpea than other studied hosts. In consonance to the present findings, a previous study[30] also recorded a positive correlation between pod fly damage and parasitism in pigeon pea. In nature, availability and suitability of host plants as well as prevailing weather parameters mainly influenced on movement of *M. vitrata*[31] and during extreme weather conditions survival of both *Maruca* larvae and its parasitoids can be supported by alternate hosts.

So far, no research works were carried out on influence of weather parameters on parasitoids of *M. vitrata* and hence the present work may form a basis for further studies. Even though a substantial number of parasitoid species against *M. vitrata*[20-26] have been reported from the earlier reports and including the present observations, they have not been successfully exploited because of only low to medium level of parasitism in the field conditions. This could be attributed to the hidden nature of feeding by *M. vitrata* larvae inside the dense webbing of flowers and pods. It diminishes the access of parasitoids for host insect identification[32] thereby safeguarded from natural enemies and these become a great challenge to management practices. Also, these parasitoids are not species specific, they might have preference to parasitize on other insects during the availability of

alternate hosts in the environment. Nevertheless, the widespread damage of *M. vitrata* on different host crops, the availability of an effective parasitoid would make a significant contribution to manage this insect pest especially to small and marginal farmers across India. However, further researches and surveys need to be carried out on these parasitoids and other unidentified parasitoids for their successful exploitation for sustainable biocontrol program of *M. vitrata.*

COI primer profile of *Bassus* sp. and braconid wasp of *M. vitrata* recorded the amplicon size of ~800 bp. In the recent past, Cytochrome Oxidase I (COI) based molecular species identification is found to be highly accurate[33]. COI gene has been used to study the phylogenetic relationship of different geographical populations of *M. vitrata* from Tropical Asia and sub-saharan Africa[34] and South India[31].

The endosymbiotic bacteria, *Wolbachia* are wide spread in many insect species especially the parasitic hymenopterans. It mainly harbours the reproductive system of insects and known to be inherited[35] and transmitted predominantly through females and affects the sex ratio of many insects. Generally, allele specific polymerase chain reaction (Standard PCR) is commonly used for the amplification of *Wolbachia* DNA from insects. The alignment sequence of larval parasitoids such as *Bassus* sp. showed maximum similarity with 49 and 14 haplotypes of *B. binominatus* and *B. dimidiator.* respectively. The braconid wasp had the maximum identity of 87.0% with the sequence of *Asobara japonica* isolate Kagoshima followed by 86.0% to *Diaeretiella rapae* Stary. Association of *Wolbachia* is common in parasitoids and some predators mainly for their parthenogenetic reproduction. Sambathkumar *et al*. (2017)[36] recorded presence of *Wolbachia* in larvae of *M. vitrata* through specific primers. Similarly, occurence of *Wolbachia* was detected in parasitoids such as, *Exorista sorbillans* Wiedemann and *Hierodulla* spp[37]. It also obtained positive reactions of *Wolbachia* primer amplification with 35 arthropod species. Infection of *Wolbachia* was also reported in many lepidopterans[38]. It was also demonstrated the *Wolbachia* causing feminization in the *Ostrinia scapulalis* Walker, induced male killing when transferred to the Mediterranean flour moth, *Ephestia kuehniella* Zeller[39].

Association of *Wolbachia* with arthropods and especially in the insects are attributed to manipulating the reproduction by acting as reproductive parasites through cytoplasmic incompatibility, feminization of males and male killing (as functional alteration to females)[40,41], parthenogenetic reproduction[42] and alteration of oogenesis[43]. In nature, the longevity and fecundity are highly related to fitness of parasitoids. In light of this, the group of maternally transmitted *Wolbachia* endosymbiont tremendously plays significantly through manipulation of the fitness of its host[44] in terms of reproduction and defense against pathogens[45]. As *Wolbachia* mainly involve in the alteration of sex ratio and generation multiplication towards female sex, it could be encouraged for the context of *M. vitrata* management. This can be achieved by maximum number of female wasp population in the environment through manipulating or exploiting the role of *Wolbachia.* This led considerable level of parasitism on *M. vitrata* larvae and thereby reduces the excess use of synthetic pesticides give less harm to the ecosystem.

Conclusion

Among different parasitoids observed on *Maruca vitrata*, *Bassus* sp. was found to be predominant and occurs year round in all host crops than other parasitoids. Larval parasitoids *Bassus* sp. and braconid wasp yielded specific fragments (~800 bp) with mitochondrial COI primer. Presence of *Wolbachia* was confirmed in all four larval parasitoids with the amplicons size of ~ 600 and 650 bp. This clearly indicated that *Bassus* spp. has close association with *M. vitrata* and further studies on the suitability and its mass culturing can be attempted for viable and sustainable management of *M. vitrata* with the help of biocontrol agents.

Acknowledgement

The first author is grateful to the INSPIRE Fellowship funded by the Department of Science and Technology, Government of India and also to ICAR.

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