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Laboratory evaluation of aqueous plant extracts against rice yellow stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera:Crambidae) and its safety to natural enemies

Sharmitha T[1*], Gailce Leo Justin C[2] and Sheeba Joyce Roseleen S[2]

[1] Department of Agricultural Entomology, Tamil Nadu Agricultural University (TNAU), Coimbatore – 641 003, Tamil Nadu, India

[2] Department of Plant Protection, Anbil Dharmalingam Agricultural College and Research Institute, TNAU, Tiruchirappalli - 620 027, Tamil Nadu, India

*Corresponding author (Email: sharmithavvta1@gmail.com)

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Abstract

Laboratory studies were conducted to evaluate the effect of aqueous extracts of pungam, tulsi, periwinkle, notchi, sweet flag and neem seed kernels on egg, larva and adult of rice yellow stem borer. The effect of botanicals on egg masses revealed the maximum ovicidal effect of reduced hatching (5.58 %) by sweet flag rhizome extract followed by NSKE (21.35 %) and pungam leaf extract (34.08 %). The maximum antifeedant effect in terms of stem protection was observed in NSKE (74.55 %) followed by pungam leaf extract (67.24 %). All the botanicals were observed to exert equal anti-oviposition effect (100 %) on adult moths of yellow stem borer except tulsi (96.67 %). The botanicals were found to be safe to the egg parasitoids *Telenomus dignus* and *Tetrastichus schoenobii* with maximum adult emergence (> 90 %) from the treated egg masses of yellow stem borer compared to control exhibiting maximum natural parasitism (93.33 %), during *Kharif*, 2018 and *Rabi*, 2019.

Keywords: Yellow stem borer, botanicals, toxicity, egg parasitoids, safety

Introduction

Rice (*Oryza sativa* L.) belonging to family Poaceae is an important grain crop and staple food for more than half of the human population (Zhang, 2019). Globally, it is the second most cultivated cereal crop next to wheat. India ranks first in area (43.79 m. ha) and second in production (109.70 MT) (Anonymous, 2018). Tamil Nadu is one of the major rice producing states in India. The rice crop is subjected to a considerable damage by nearly 300 species of insect pests; among them only 23 species are serious pests of rice (Pasalu and Katti, 2006). In India, out of the total loss incurred by different insect pests of paddy, 30 per cent damage is done by stem borer alone (Krishnaiah and Varma, 2012). The yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) (Lepidoptera: Crambidae) is widely distributed and the most predominant species of stem borer in rice ecosystem (Reuolin and Soundararajan, 2019).

Globally, rice stem borer management accounts for 50 per cent of the insecticides used in rice fields (Huesing and English, 2004). Overreliance on synthetic pesticides causes ecological adversity and health related problems (Carvalho, 2017). It has also led to an exponential increase in the number of insect species developing resistance to insecticides (Sparks and Nauen, 2015) and destruction of population of beneficial insects (Jafar *et al.*, 2013). Rice ecosystem harbours a wide range of natural enemy complex (Singh and Kumawat, 2020). Parasappa (2017) reported a significant positive correlation of YSB population with the hymenopteran parasitoids of rice ecosystem. The extent of natural egg parasitism of yellow stem borer in rice ecosystem has been reported to a maximum of 95.00 per cent (Lakshmi *et al.*, 2010). However, a significant reduction in predators and adults of the egg parasitoids *Trichogramma sp.*, *Telenomus sp.* and *Tetrastichus sp.* has been reported in the insecticide treated rice plots compared to untreated plots (Rahaman and Stout, 2019). These reports suggest the potential role of indigenous parasitoids and the need for their conservation *in situ* for suppressing the population of YSB. The use of botanicals in pest management is the best alternative to insecticides as they are safe to environment and compatible with natural enemies and other beneficial organisms. With this in view, a preliminary study was conducted in the laboratory to evaluate the detrimental effect of botanicals on different stages of yellow stem borer and their safety to the egg parasitoids of YSB.

Materials and Methods

Field study

Natural parasitism of yellow stem borer egg mass

A field trial was conducted at the experimental farm of Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli District, Tamil Nadu, in a Randomised Block Design during *Kharif*, 2018 and *Rabi*, 2019 with rice cv. TRY 3. The standard agronomic practices recommended by Tamil Nadu Agricultural University were adopted except the plant protection measures. The egg masses of yellow stem borer (YSB) were collected thrice per month with 10 days interval (30 egg masses/replication) during *Kharif*, 2018 and *Rabi*, 2019 from the field and kept in the petri plates with moist filter paper to avoid drying of leaves and observed for the emergence of the adult parasitoids. Once emergence was completed, the emerged adult parasitoids were observed under the stereo-zoom microscope to identify the species. The extent of parasitism of egg masses of YSB was worked out as given under (Vennila *et al.*, 2018)

$$\text{Parasitism (\%)} = \frac{\text{No.of parasitized egg masses}}{\text{No.of sample egg masses}} \times 100$$

Laboratory studies

Mass rearing of yellow stem borer

The YSB was reared following the methodology described by Subashrao (2009) with slight modifications. The YSB adults collected from the field using sweep net were sexed and caged by providing rice leaves for oviposition by female. The lower portion of rice leaves (cv. TN 1) were kept in small glass vials containing water, so as to keep the leaves fresh and turgid for longer period. A small piece of cotton, duly saturated with five per cent sugar solution was provided in the cage as food for adults. The egg masses laid on the leaves were taken out daily along with leaf and kept in the petri dishes with filter paper and stored at 15˚C. All the egg masses collected and stored were taken out on the same day and placed in petri dishes with moist filter paper to obtain uniform hatching of the larvae.

The newly hatched larvae were transferred to the tender pieces of rice stem with the help of soft camel hair brush. The hollow stems of rice from the top portion of the plant with leaf sheaths were selected for the neonate larvae and the hollow stems from the bottom portion of the plant were given as the larva reached the third instar. The hollow stems were cut such that each contained a node on both the end. The bases of rice stems were wrapped with foam and placed horizontally in a tray with thin film of water to enable the development of roots from both the nodes to keep the stems fresh and turgid for longer period. Then the larvae (10 neonate/ cut stem) were introduced into the small slit made at the centre of the rice stem with the help of camel hair brush. The top of the tray was covered with muslin cloth and fastened with rubber band to prevent the larvae from escaping and to keep the stems fresh and turgid by maintaining the humid condition in the tray. The larvae were transferred to fresh rice stems at two days interval until they pupated. After pupation, each stem was split opened from the middle of the stem up to the site of pupation. This facilitates easy emergence of the adult from the pupa in the laboratory. The stems with pupae were kept inverted in a glass vial with water and kept inside the adult emergence cage to keep the stems moist until the adults emerged from the pupae. The emerged adults were used for subsequent rearing. The reared eggs, larvae and adults were utilised for the laboratory experiments.

Effect of botanicals on adult emergence of egg parasitoids of YSB

The naturally parasitised egg masses of YSB were collected from the field and kept in Petri dishes lined with moist filter paper to avoid drying of leaves. The egg masses were then treated by dipping in the aqueous extract of botanicals for five seconds. Water was used as untreated check. The treatments were replicated thrice and the treated egg masses were shade dried for 15 min. and then kept in Petri dishes at the rate of three egg masses per treatment and sealed. The egg masses were observed for the emergence of the adult parasitoids. The experiment was terminated on completion of adult emergence in the control. The egg masses were later dipped in 70 per cent ethanol and the hairs were removed with needle. The eggs were then separated out with the help of a fine camel hair brush and needle and the number of hatched and unhatched eggs as well as emerged and unemerged adults were counted under a stereo zoom microscope. The emergence of each species of egg parasitoid from the egg masses was worked out by the formula given by Vennila *et al.*, (2018).

$$\text{Adult emergence (\%)} = \frac{a}{a+b} \times 100$$

Where, a- number of parasitoids emerged, b- number of parasitoids un-emerged

Effect of botanicals on different stages of YSB

Preparation of botanical extracts

The aqueous extracts of botanicals were prepared following the standard procedure described. The leaves of pungam, *Millettia pinnata* (L.) Panigrahi; tulsi, *Ocimum sanctum* L.; periwinkle, *Catharanthus roseus* (L.) G. Don; notchi, *Vitex negundo* L.; rhizome of sweet flag, *Acorus calamus* L. and neem seed kernels were collected, washed and cut into small pieces. The cut leaves (Jazzar and Hammad, 2003), rhizomes (Yasodha and Natarajan, 2007) and neem seed kernels (Venkat Reddy *et al.*, 2012) were ground separately and mixed with distilled water at 5 per cent concentration *i.e.*50 g leaves/rhizomes/neem seed kernels per litre of water, soaked overnight, then filtered through muslin cloth. Tween 20 (0.5 %) was added as a surfactant.

Ovicidal activity of botanicals on YSB egg mass

Rice leaves with egg masses (3 days old) from the laboratory rearing were collected and immersed for five seconds in each botanical (Temerak, 2006). The treated egg masses were allowed to shade dry for 15 minutes and placed in Petri dishes lined with moistened filter paper to avoid drying of leaves and was sealed and observed for seven days. Three replications were maintained per treatment. The experiment was terminated after hatching of all the eggs in the control and the egg masses were dipped in 70 per cent ethanol. After five minutes the egg masses were taken out and the hairs were removed with needle under microscope. The hatched and unhatched larvae/ eggs were counted under a stereo zoom microscope. The ovicidal action was measured by calculating the hatching percentage (Heinrichs,1981).

Larval hatching (%) = [(No. of larva hatched)/ (No. of larva hatched + No. of eggs unhatched)] ×100.

Antifeedant effect of botanicals on YSB larvae

The larval bioassay with botanicals was conducted by following the methodology suggested by Heinrichs (1981). Cut stalks of rice culms from the base of the plant at a length of about 75 mm with nodes at both the ends were chosen and placed in a glass vial/ test tube each, with water to prevent drying of the stalk and by the rooting of the node at the bottom of the stalk. The rice culms were dipped in the botanicals for five seconds for proper soaking. At one day after treatment, both the ends of the culm were sealed by dipping them in hot paraffin wax to seal the ends and were infested with a third instar larva. Each vial/ test tube served as a replication. Cotton soaked with water was placed at the top over the end of the tube to prevent the drying of stalk. Two days after infestation, the cut stems were dissected and the length of stem feeding was measured (Islam *et al.*, 2013). The stem protection over control (%) was worked out by the formula,

$$A (\%) = [(B – C) / (100 – C)] \times 100$$

Where, A - stem length protection over control (%),

B- stem length protection in treatment (%), C- stem length protection in control (%)

Oviposition deterrent effect of botanicals on YSB adults by free choice test

Rice leaf tips (cv. TN1) were dipped in the botanicals for five seconds for uniform soaking and shade dried. The treated leaf tips were kept in glass vials with water to prevent the drying of leaves. Three replications were maintained. Water was used as untreated check. Food source of five per cent honey solution soaked in cotton wool was provided for the stem borer moths. Twenty adults of YSB (female) were released into the cage, 24 h after treatment. Oviposition was observed in the leaves after 24 h of adult release. The anti-ovipositional effect was calculated for each treatment by using the following formula:

Oviposition deterrent effect (%) = [No. of eggs laid on untreated surface / Total no. of eggs laid (treated + untreated surface)] × 100

When mortality (%) was observed in the control for the laboratory bioassays, the value was corrected by the following Abbot's formula (Abbott, 1925):

Corrected mortality (%) = [(Test mortality (%) – Control mortality (%)) / (100- Control mortality)] × 100

Results and Discussion

Safety of botanicals to egg parasitoids of YSB

The natural parasitism of egg mass of YSB was maximum in January 2019 (93.33 %), which was on par with the parasitism in February 2019 (90.00 %) and in December 2018 (80.00 %) during *Rabi*, 2019 (Table 1). The parasitism of egg masses during *kharif* 2018 was minimum in August (20.00 %) which subsequently increased in September 2018 (33.33 %) and October 2018 (40.00 %).

Table 1: Natural parasitism of egg mass of yellow stem borer, *S. incertulas*

Season	Month	Egg mass (no.)*		Parasitism (%)*
		Sampled	Parasit-ised	
Kharif, 2018	July	0.00	0.00	0.00 (0.91) e
	August	30.00	6.00	20.00 (26.67) d
	September	30.00	10.00	33.33(35.26) cd
	October	30.00	12.00	40.00 (39.23) c
Rabi, 2019	November	30.00	13.00	43.33 (41.17) c
	December	30.00	24.00	80.00 (63.44) ab
	January	30.00	28.00	93.33 (75.03) a
	February	30.00	27.00	90.00 (71.57) ab
	March	30.00	22.00	73.33 (58.91) b
Total	-	240.00	141.00	63.08
SEd	-	-	-	8.07
CD (p=0.05)	-	-	-	16.96

*Mean of three replications; Figures in parentheses are arcsine transformed values; In a column, means followed by similar letter(s) are not statistically different (p=0.05) by LSD

Telenomus dignus (Gahan) and *Tetrastichus schoenobii* (Ferriere) were the two parasitoids observed in the egg masses collected from the experimental plot. Earlier, Varma *et al.,* (2013) reported maximum parasitisation of YSB egg masses by *Telenomus sp.*, while, Manjunath (1990) observed that maximum parasitic potential in YSB egg mass parasitised by *T. schoenobii* alone, followed by *T. dignus* and *T. schoenobii* in combination.

Observations on safety to egg parasitoids revealed that, the botanicals *Millettia*, *Acorus, Vitex, Catharanthus, Ocimum* and NSKE at a concentration of five per cent were safe to egg parasitoids *T. dignus* and *T. schoenobii*, which was evident by above 90 per cent emergence of adult parasitoids from the treated egg masses (Table 2 & Figure 1)**.** This is in alignment with the previous findings of the repellent effect of neem cake and neem oil on YSB moths with a significant reduction in oviposition but without reducing the extent of egg parasitism by the parasitoids *T. dignus* and *T. schoenobii* (Manju and David, 2004).

Table 2: Laboratory evaluation on the safety of botanicals on egg parasitoids of yellow stem borer, *S. incertulas*

Treatments	Conc. (%)	Parasitoid emergence (%)	
		T. dignus	*T. schoenobii*
T1 - *Millettia* leaf extract	5	90.85 (72.39) d	91.33 (72.87) c
T2 – NSKE	5	90.09 (71.65) d	90.38 (71.93) c
T3 - *Acorus* rhizome extract	5	91.64 (73.20) cd	92.22 (73.81) bc
T4 - *Vitex* leaf extract	5	93.67 (75.43) bc	92.12 (73.70) bc
T5 - *Ocimum* leaf extract	5	94.80 (76.82) bc	93.33 (75.03) bc
T6 - *Cathranthus* leaf extract	5	96.33 (78.96) b	97.70 (81.28) ab
T7 – Control	-	100.00 (89.10) a	100.00 (89.10) a
SEd		1.59	4.93
CD (p=0.05)		3.41	10.57

*Mean of three replications, Figures in parentheses are arcsine transformed values
In a column, means followed by similar letter(s) are not different statistically (p=0.05) by LSD

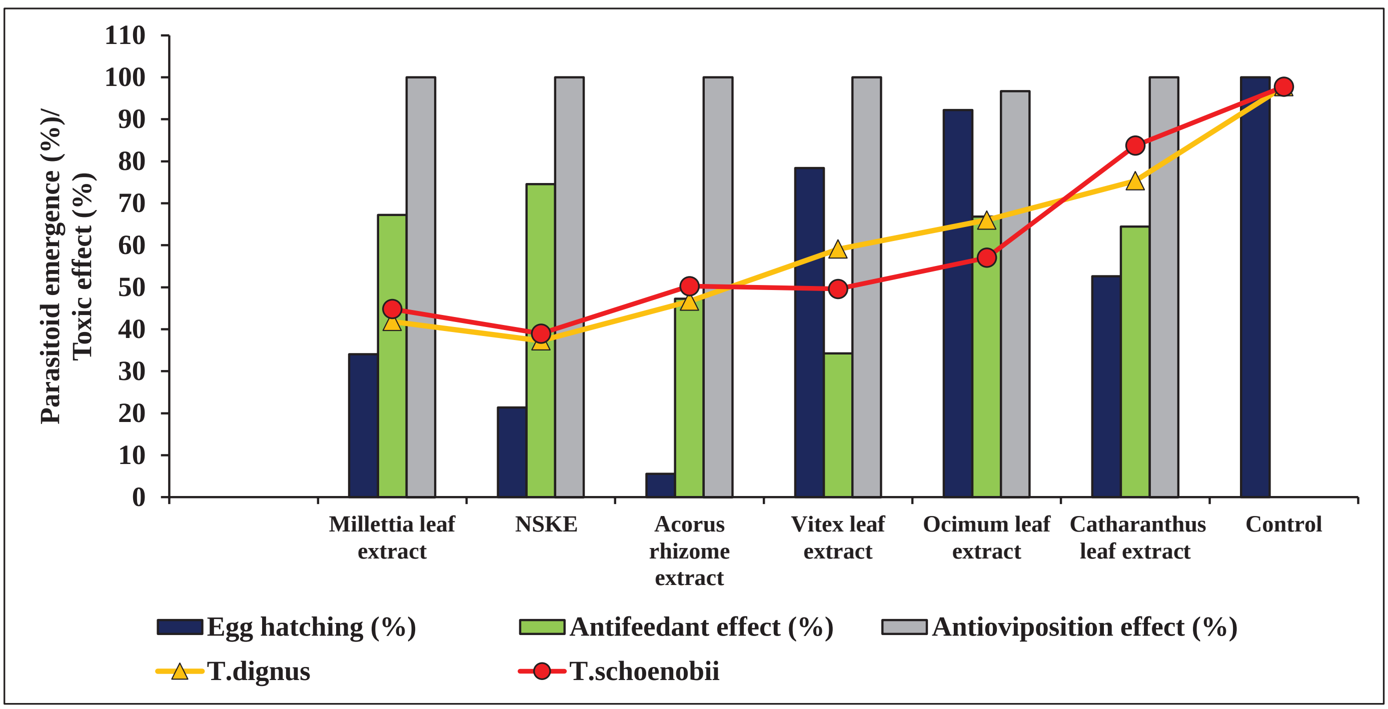

Figure 1: Effect of botanicals on the different stages of yellow stem borer and its safety to egg parasitoids

Evaluation of botanicals on different stages of YSB

Among the botanicals evaluated, *Acorus* rhizome extract exerted maximum ovicidal action which was evident by significantly low percentage of larval hatching of YSB (5.58 %), followed by NSKE (5 %) showing 21.35 per cent hatching and *Millettia* leaf extract (5 %) with 34.08 per cent hatching of YSB eggs (Table 3 & Figure 1). Aqueous extract of

A. calamus was found to exhibit maximum ovicidal action (38.89 % egg hatch) on *Plutella xylostella* (L.) (Matharu and Mehta, 2017) while Lall *et al.*, (2014) reported maximum reduction in egg hatching of egg masses of *Spodoptera litura* (Fabricius) treated with NSKE. *V. negundo* was reported to exhibit 52.02 per cent ovicidal activity against *S. litura* egg mass (Arivoli and Tennyson, 2013), which is in alignment with the least effect of *Vitex* on YSB eggs (78. 39 % YSB larval hatching). The ovicidal effect of *Millettia* on YSB is supported by the 80 per cent mortality by aqueous extract of *Pongamia glabra* Vent. on eggs of *Oligonychus coffeae* Nietner (Vasanthakumar *et al.*, 2012).

Table 3: Laboratory evaluation of botanicals on different stages of rice yellow stem borer, *S. incertulas*

Treatments	Conc. (%)	Larval hatching (%)*	Antifeedant effect (%) *	Antioviposition effect (%)*
T_1 – *Millettia* (Pungam) leaf extract	5	34.08 (35.72)c	67.24 (55.09)b	100.00 (89.38)a
T_2 – Neem Seed Kernel Extract (NSKE)	5	21.35 (27.52)b	74.55 (59.70)a	100.00 (89.38)a
T_3 - *Acorus* rhizome extract	5	5.58 (13.66)a	47.25 (43.42)c	100.00 (89.38)a
T_4- *Vitex* leaf extract	5	78.39 (62.30)e	34.22 (35.80)d	100.00 (89.38)a
T_5 - *Ocimum* leaf extract	5	92.20 (73.78)f	66.81 (54.82) b	96.67 (79.49)b
T_6 - *Catharanthus* leaf extract	5	52.65 (46.52)d	64.42 (53.38)b	100.00 (89.38)a
T_7 – Control	-	100.00 (87.14)g	0.00 (2.87)e	0.00 (0.63)
SEd		2.37	1.54	2.08
CD (p=0.05)		5.07	3.31	4.47

*Mean of three replications; Figures in parentheses are arcsine transformed values
In a column, means followed by similar letters are not different statistically (p=0.05)

The present study revealed that the antifeedant effect was maximum in NSKE with 74.55 per cent stem protection from YSB larval feeding followed by *Millettia* leaf extract with 67.54 per cent stem protection, over control (Figure 1). Maximum anti-oviposition effect (100 %) was observed in the botanicals *Millettia*, NSKE, *Acorus*, *Vitex* and *Catharanthus* followed by *Ocimum* (96.67 %) (Table 3). Chakraborty (2011) reported least damage of stem borer with NSKE (5%) and maximum damage with *V. negundo* extract (5%). Rao *et al.*, (2002), reported antifeedant effect (80%) against third instar larvae of *Earias vitella* (Fab.) on combined application of neem, sweet-flag and pungam extracts (1:1:1). The combination of aqueous extracts of NSKE (5%) and *A. calamus* (5%) exhibited strong ovicidal action and ovipositional deterrence against *Leucinodes orbonalis* (Guenee) (Yasodha and Natarajan, 2007). NSKE (5%) has been reported as the suitable alternative to synthetic insecticides for management of rice stem borers (Ogah *et al.*, 2011).

Conclusion

Management of YSB dead heart and white ear damage is difficult as the larval stage is concealed inside the stem. Hence, management of this pest is easy and effective at the egg stage which prevents the further development of dead heart and white ear that leads to yield loss. Natural parasitism of YSB egg masses was observed at a maximum rate which leads to the effective control of YSB at the egg stage itself. Hence, keeping in view the natural occurrence of egg parasitoids in rice ecosystems, measures must be taken to avoid insecticide spray to conserve them. Thus, the application of efficacious botanical derivatives like NSKE (5 %), *Acorus* rhizome extract (5 %) and *Millettia* leaf extract (5 %) with ovicidal and repellent action on YSB can be developed as an alternative tool to insecticides for successful, eco-friendly and cost effective management strategy with safety to the egg parasitoids predominantly found in the rice ecosystem.

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