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Evaluation of bio-efficacy of new generation insecticides, botanicals and microbial insecticides on leaf webber of amaranth

Pooru Muralikrishna, Thomas Biju Mathew, Ambily Paul and Nithya PR

Abstract
Hymenia recurvalis is a major leaf eating pest on vegetable amaranth. A laboratory study was conducted on evaluation of bio-efficacy of new generation insecticides against *H. recurvalis*. New generation insecticides (chlorantraniliprole - 0.006%, novaluron - 0.015%, flubendiamide - 0.0096%, spinosad - 0.015%,emamectin benzoate - 0.002%, indoxacarb - 0.015%, thiacloprid - 0.036%, and fipronil - 0.01%), botanicals (NSK 5 ml L^{-1} and oxuron 5 ml L^{-1}) and microbial insecticide, *Bacillus thuringiensis* kurstaki were evaluated *in vitro.* Chlorantraniliprole 0.006%, flubendiamide 0.0096%, emamectin benzoate 0.002%, indoxacarb 0.015%, thiacloprid 0.036%, fipronil 0.01% and B.t k 5 ml L^{-1} were found to be effective under laboratory conditions and field studies were conducted using these effective insecticides. Under field studies, flubendiamide 0.0096% was found effective in managing *H. recurvalis* with least toxicity to non-target organisms such as spiders.

Keywords: *Hymenia recurvalis*, bio-efficacy, safer insecticides, chlorantraniliprole, flubendiamide, *Bacillus thuringiensis*

1. Introduction
Red Amaranth (*Amaranthus tricolor* L.) is a leafy vegetable popular throughout the tropics and in many warm temperate regions. It is regarded as a good source of vitamins including vitamin A, vitamin K, vitamin B6, vitamin C, riboflavin. Amaranth is also a rich source of minerals *viz*., calcium, iron, magnesium, phosphorus, potassium, zinc, copper and manganese. Red amaranth is one of the important leafy vegetable grown in India and is said to be the native of India. This leafy vegetable is very popular in South India because of its nutritious nature and much less agronomic practices for cultivation. Amaranth is raised around the year in the paddy lowlands, garden lands, and homesteads in Kerala, where land area total is less compared to other states.

Insects like leaf web bers, green grasshopper and tobacco caterpillar infest and devour the leaves of amaranth, causing severe yield losses (Nair, 1975) [1]. Leaf webber, *H. recurvalis* is an important pest of spinach, sugarbeet and other crops in central and southern Japan (Yamada *et al.,* 1979) [2]. Larvae of *H. recurvalis* destroy the leaves by webbing them together and feeding from inside for five to seven generations per year (Pande, 2009) [3]. This pest has been regarded as a dangerous defoliator for the crop, sugar beet too (Cook and Scott, 1993) [4]. Similarly, in Egypt, this pest became a menace to sugar beet plants for the first time in 2001 (El-Gendi *et al*., 2003) [5]. In the past few years, this pest has become a major defoliator of amaranth in Kerala.

Plant protection measures against this pest, especially in commercial cultivation of amaranth, has largely been based on chemical pesticides. Dales (1996) [6] put forward the health risks and environmental pollution caused by the use of synthetic insecticides. Since, insects and other animals have similar tissue, reproductive, hormonal and nerve systems, these compounds have a prospective effect on non-target organisms including human beings (PPDB, 2014) [7]. The ready consumable nature of this vegetable without much processing demands the use of much safer chemicals for successful management of pests. Besides that intensive use of conventional insecticides resulted in several problems *viz.,* pesticide residues, pest resurgence, and resistance in pest population. This scenario necessitated the discovery and use of new chemical insecticides which are more effective in pest management, safer for humans and much less toxic to our ecosystem (Korrat *et al*., 2012) [8].

Replacement of conventional insecticides by novel insecticides is thus the need of the hour. In this context, the current investigation was undertaken to study the effect of new generation insecticides, botanicals and microbial insecticides in managing leaf webber of amaranth.

2. Materials and methods

H. recurvalis were reared in laboratory conditions by adopting standard method (Shirai, 2006) [9]. Different instar larvae of *H. recurvalis* were collected from fields of instructional farm, College of Agriculture, Vellayani. They were reared in the laboratory in separate troughs up to the adult stage. These adults were reared in a cage (1.6X 1.0 X 1.18 m^2) with two windows at each side, one to release the moths and another to introduce pots of plants and for collecting leaves with egg mass. The entire cage had iron mesh for easy observation. Inside the cage, four potted amaranth plants at the age of 20-25 days were kept and moths were released for egg-laying. They fed with 5% diluted honey solution, using cotton buds dipped in 5% honey and hang from the roof of cage @ 10 buds per cage. The egg masses obtained from the plants were kept in Petri dishes with fresh leaves. The second instar larvae were taken out and used for different experiments.

The different treatments used were chlorantraniliprole - 0.006% (Coragen), flubendiamide - 0.0096% (Fame), spinosad - 0.015% (Tracer), emamectin benzoate - 0.002% (Proclaim), indoxacarb - 0.015% (Avaunt), thiacloprid - 0.036% (Alanto), fipronil- 0.01% (Regent), *Bacillus thuringiensis* kurstaki - 5 ml L^{-1} (Abtek), Oxuron - 5 ml L^{-1}, neem seed kernel extract - 5% and malathion - 0.1% (Celthion) were sprayed on amaranth using hand sprayer. Leaves with uniform size were collected from randomly selected plants from this bulk crop. These leaves were placed in Petri plates at the rate of one leaf per Petri plate. Second instar larvae of *H. recurvalis* were released separately into Petri plates containing treated leaves (Tukaram *et al.,* 2014) [10]. Ten larvae were released per Petri plate and observations were taken at six hours interval. Larvae were considered dead when there was no movement when disturbed with Camlin zero brush. Dead larvae were counted and discarded after every observation. The percent mortality was corrected by using Abbot's formula (Abbot, 1925) [11].

Five insecticides found as effective in laboratory were tested in field conditions along with insecticides check and an untreated check against *H. recurvalis*. The treatments selected for field evaluation are mentioned in Table 2. The experiments were laid out in a randomized block design (RBD) with four replications. Plots of 2 x 2 m were prepared with 30 cm ridges between plots. All the management practice except the plant protection against insect pests in amaranth were followed as per the recommended package of practices of Kerala Agricultural University (KAU, 2011) [12]. Four sprayings were done at seven days interval. First spraying was done on the seventh day after sowing. Observations were taken at first, third, fifth day after every spraying as post-treatment count and one pre-treatment count before every spray. Observations were taken for the number of infested leaves out of total leaves per plant in five plants, number of larvae before and after the treatment in five plants, number of natural enemies before and after the treatments.

3. Results and Discussion

Preliminary evaluation of selected new generation chemical insecticides, botanicals and microbial insecticide against *H. recurvalis* was conducted *in vitro.* Cent percent mortality of treated larvae was recorded at six hours after release in fipronil 0.01% whereas 55.30 percent mortality in malathion 0.1% (insecticidal check) treated larvae (Table 1). Majula and Kotikal (2015a) [13] also reported the efficacy of fipronil against larvae of *H. recurvalis*.

With the advent of exposure time (18 hours after release,), cent percent mortality was noticed in emamectin benzoate 0.002%, indoxacarb 0.015%, thiacloprid 0.036%, fipronil 0.01%, and *B. thuringiensis* kurstaki - 5 ml L^{-1}. The insecticidal check, malathion 0.1% too recorded 100% mortality after 18 hours of treatment. The cessation of feeding and movement arrest in case of *B. thuringiensis* treated larvae were taken into account while mortality assessment. Aswal (2012) [14] reported an 85 percent reduction in larval population of *H. recurvalis* when treated with *B. thuringiensis.* Similar results were obtained with *B. thuringiensis* against *H. recurvalis* in previous studies too (Leena *et al.*, 2005) [15]. El-Sayed (2017) [16] reported emamectin benzoate as the most potent among the tested new generation insecticides at both acute and chronic levels. Effectiveness of emamectin benzoate in managing *H. recurvalis* was reported by Majula and Kotikal (2015b) [17] as faster larval mortality was found with emamectin benzoate than indoxacarb.

By 36 hours after treatment, cent percent mortality was recorded with chlorantraniliprole 0.006%, flubendiamide 0.0096% and spinosad 0.015% also. Even though flubendiamide 0.0096% and chlorantraniliprole 0.006% treated larvae took 36 hours, to cause cent percent mortality, all larvae in these treatments ceased feeding on amaranth leaves within six hours after exposure because of the specific mode of action of these diamides. Masanori *et al*. (2005) [18] reported the efficacy of flubendiamide in successfully managing lepidopteran insects. Diamide insecticides significantly reduced the carbohydrate content of the host plant, associated with general disturbances in carbohydrates metabolism, expressed by significant inhibition of activities of digestive hydrolyzing enzymes (Rashwan, 2013) [19].

A field experiment was laid out to evaluate the efficacy of the best five treatments. Treatments used in field analysis are mentioned in Table 2. The results indicated that all treatments were effective in controlling *H. recurvalis* when compared with those of untreated control. On the third day after treatment, no population observed in all four fipronil 0.01% treated plots (Table 2). Comparatively less reduction was observed in flubendiamide 0.0096% treated plots (0.93) and was comparable to the insecticidal check malathion (0.10%). At five days after treatment, there was no significant difference between the treated plots. All plots did not show any significant difference with untreated control after the first spray. After second spray except flubendiamide 0.0096% all the treatments were superior over insecticidal check and flubendiamide 0.0096% was statistically on par to insecticidal check malathion 0.1% (Table 2). Among all treatments, no treatment found as below than insecticidal check, malathion 0.1% ineffectiveness on mean population of *H. recurvalis.* While in percentage of infestation also there was no significant difference among all treated plots (Table 3). The present findings on the effectiveness of indoxacarb, emamectin benzoate, fipronil, flubendiamide, novaluron, and malathion were in agreement with the findings of Majula and Kotikal, (2015a) and Majula and Kotikal (2015b). Even though flubendiamide 0.0096% and chlorantraniliprole 0.006% treated larvae took 36 hours, to cause cent percent mortality, all larvae in these treatments have stopped feeding on amaranth leaves within six hours after exposure because of the specific mode of action of these diamides. This may be attributed to the exceptional insecticidal activity of diamides to a range of lepidopteran pests as well as many other insect orders, *viz.,* Coleoptera, Diptera, Isoptera and Hemiptera (Sattelle *et al*., 2008; Lahm *et al*., 2009) [20, 21]. Applications of diamide insecticides led to a significant reduction in carbohydrate content, associated with general disturbances in carbohydrates metabolism, as expressed by significant inhibition of digestive hydrolyzing enzymes activities (Rashwan, 2013).

Effect of the tested insecticides against non-targeted organisms such as natural enemies were also assessed during the current investigation. In the case of toxicity of tested insecticides on the spider population in amaranth ecosystem, less toxicity was observed in flubendiamide 0.0096% treated plots (1.23-2.19). The number of spider population in flubendiamide treated plots were more or less similar to the population observed in untreated control plots (1.23-2.74) (Table 4). Safety of diamides to spiders were also reported by (Rajavel, 2011) [22].

4. Conclusion

In the current scenario, where people have become more conscious about the ill effects of excessive use of chemical pesticides, better alternatives for eco-friendly pest management is the need of the hour. As chemical pesticides cannot be fully wiped off from the management practices, our investigation proposes green chemicals *viz.*, Chlorantraniliprole 0.006% and Flubendiamide 0.0096% for the safe management of insect pests in a widely consumable leafy vegetable, amaranth.

Table 1: Effect of new generation insecticides, botanicals and microbial insecticides on the mortality of *H. recurvalis* under laboratory conditions

Treatments	Mean percentage mortality of *Hymenia recurvalis* when observed at					
	6 HAS	**12 HAS**	**18 HAS**	**24 HAS**	**30 HAS**	**36 HAS**
T1-Chlorantraniliprole 18.5 SC - 0.006%	5.57^{e} (8.81)	11.11^{g} (16.45)	11.11^{e} (16.45)	50 .00^{bc} (45.00)	88.89^{bc} (62.18)	100.00^{a} (88.83)
T2- Novaluron 10 EC - 0.015%	33.33^{d} (34.78)	50.00^{cd} (45.00)	77.78^{b} (62.18)	88.89^{a} (62.18)	100.00^{a} (88.83)	100.00^{a} (88.83)
T3- Buprofezin 25 SC - 0.03%	27.78^{d} (31.54)	27.78^{ef} (37.54)	38.89^{cd} (38.51)	61.11^{b} (51.49)	83.33^{bc} (62.18)	55.55^{c} (48.24)
T4-Flubendiamide 39.35 SC - 0.0096%	5.57^{e} (8.81)	33.33^{de} (35.26)	44.44^{cd} (41.74)	61.11^{b} (51.49)	94.44^{ab} (81.19)	100.00^{a} (88.83)
T5-Spinosad 45 SC - 0.015%	11.11^{de} (16.45)	27.78^{ef} (37.54)	50 .00^{c} (45.00)	66.67^{b} (55.21)	77.78^{cd} (62.18)	100.00^{a} (88.83)
T6- Emamectin benzoate 1 WG - 0.002%	83.33^{abc} (69.74)	94.44^{a} (81.19)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)
T7- Indoxacarb 14.5 SC - 0.015%	94.44^{ab} (81.10)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)
T8- Thiacloprid 21.7 SC - 0.036%	88.89^{abc} (73.54)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)
T9- Fipronil 5 SC - 0.01%	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)
T10- *Bacillus thuringiensis* kurstaki - 5 ml L^{-1}	66.67^{c} (54.73)	88.89^{ab} (62.18)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)
T11-Oxuron - 5 ml L^{-1}	16.67^{de} (16.45)	27.78^{ef} (37.54)	44.44^{cd} (41.74)	55.56^{b} (48.24)	72.22^{d} (58.45)	83.33^{b} (9.18)
T12 -Neem Seed Kernel Extract - 5%	5.57^{e} (8.81)	16.67^{fg} (24.09)	27.78^{d} (37.54)	33.33^{c} (35.26)	38.89^{e} (38.51)	55.56^{c} (62.18)
T13- Malathion 50 EC – 0.1%	55.30^{d} (34.78)	66.66^{bc} (54.73)	88.89^{b} (62.18)	100.00^{a} (88.83)	100.00^{a} (88.83)	100.00^{a} (88.83)
T14– Untreated	0.00^{e} (1.17)	0.00^{h} (1.17)	5.57^{e} (8.81)	5.57^{d} (8.81)	11.11^{f} (16.45)	16.67^{d} (24.09)
CD (0.05)	(18.927)	(12.159)	(11.853)	(11.387)	(11.671)	(5.791)

HAS= Hours After Spraying

Values shown in parentheses are Arc sin transformed values

Table 2: Evaluation of new generation insecticides on *H. recurvalis* population under field conditions

Treatments	Mean Population Of *Hymenia recurvalis* larvae*															
	FIRST SPRAY				SECOND SPRAY				THIRD SPRAY				FOURTH SPRAY			
	PTC	1DAT	3DAT	5DAT	PTC	1DAT	3DAT	5DAT	PTC	1DAT	3DAT	5DAT	PTC	1DAT	3DAT	5DAT
T1- Novaluron 10 EC – 0.015%	10.99 (3.46)	1.96^{bc} (1.72)	0.72^{bc} (1.31)	0.46^{b} (1.21)	0.72^{b} (1.31)	0.46^{b} (1.21)	0.72^{b} (1.31)	0.46^{b} (1.21)	0.72^{b} (1.31)	0.46^{b} (1.21)	0.22^{b} (1.10)	1.12^{bc} (1.45)	1.36^{bc} (1.54)	0.72^{b} (1.31)	0.46^{b} (1.21)	1.36^{b} (1.54)
T2- Flubendiamide 39.35 SC- 0.0096%	11.99 (3.60)	2.97^{b} (1.99)	0.93^{b} (1.39)	0.46^{b} (1.21)	0.46^{b} (1.21)	0.46^{b} (1.21)	0.93^{b} (1.39)	0.46^{b} (1.21)	0.46^{b} (1.21)	0.46^{b} (1.21)	0.22^{b} (1.10)	1.40^{b} (1.55)	1.69^{b} (1.64)	0.65^{b} (1.29)	0.46^{b} (1.21)	0.99^{b} (1.41)
T3- Emamectin benzoate 1 WG -	12.24 (3.64)	1.23^{cd} (1.49)	0.65^{bc} (1.29)	0.65^{b} (1.29)	0.65^{b} (1.29)	0.22^{b} (1.10)	0.65^{b} (1.29)	0.87^{b} (1.37)	0.65^{b} (1.29)	0.22^{b} (1.10)	0.22^{b} (1.10)	0.65^{bcd} (1.29)	0.93^{bc} (1.39)	0.22^{bc} (1.10)	0.00^{c} (1.00)	0.65^{b} (1.29)

0.002%																
T4- Fipronil 5 SC – 0.01%	11.47 (3.53)	0.40^{d} (1.18)	0.00^{c} (1.00)	0.00^{b} (1.00)	0.00^{b} (1.00)	0.00^{b} (1.00)	0.22^{b} (1.10)	0.22^{b} (1.10)	0.32^{b} (1.12))	0.00^{b} (1.00)	0.00^{b} (1.00)	0.22^{cd} (1.10)	0.46^{c} (1.21)	0.00^{c} (1.00)	0.00^{c} (1.00)	0.93^{b} (1.39)
T5- Indoxacarb 14.5 SC – 0.015%	11.93 (3.60)	0.93^{cd} (1.39)	0.46^{bc} (1.21)	0.22^{b} (1.10)	0.46^{b} (1.21)	0.22^{b} (1.10)	0.46^{b} (1.21)	0.22^{b} (1.10)	0.46^{b} (1.21)	0.22^{b} (1.10)	0.00^{b} (1.00)	0.00^{d} (1.00)	0.65^{c} (1.29)	0.00^{c} (1.00)	0.00^{c} (1.00)	0.65^{b} (1.29)
T6-Malathion 50 EC (check) – 0.1%	11.77 (3.57)	1.69^{c} (1.64)	0.93^{b} (1.39)	0.65^{b} (1.29)	0.65^{b} (1.29)	0.65^{b} (1.29)	0.93^{b} (1.39)	0.65^{b} (1.29)	0.65^{b} (1.29)	0.65^{b} (1.29)	0.22^{b} (1.10)	0.46^{bcd} (1.21)	0.65^{c} (1.29)	0.22^{bc} (1.10)	0.22^{bc} (1.10)	0.46^{b} (1.21)
T7- Untreated	9.49 (3.24)	10.68^{a} (3.41)	11.60^{a} (3.55)	12.10^{a} (3.62)	12.38^{a} (3.66)	12.38^{a} (3.66)	11.60^{a} (3.55)	12.10^{a} (3.62)	12.38^{a} (3.66)	12.38^{a} (3.66)	12.38^{a} (3.66)	11.67^{a} (3.56)	14.37^{a} (3.92)	14.37^{a} (3.92)	14.94^{a} (4.00)	14.94^{a} (4.00)
CD (0.05)	NS	(0.344)	(0.361)	(0.372)	(0.324)	(0.317)	(0.361)	(0.372)	(0.324)	(0.317)	(0.245)	(0.369)	(0.343)	(0.258)	(0.204)	(0.447)

*Mean number of larvae observed in 5 plants; PTC- Pre Treatment Count; DAT- Days After Treatment; Values shown in parentheses are √x+1 transformed values

Table 3: Effect of pesticides on the extent of infestation of amaranth plants by *H. recurvalis* after different sprays

Treatments	Percentage of leaves infested after different sprays			
	First spray	**Second spray**	**Third spray**	**Fourth spray**
T1- Novaluron 10 EC – 0.015%	16.14 (3.38)	6.53^{b} (2.58)	7.48^{b} (2.72)	6.19^{b} (2.53)
T2- Flubendiamide 39.35 SC – 0.0096%	15.62 (3.34)	3.25^{b} (1.87)	4.01^{b} (2.12)	4.46^{b} (2.17)
T3- Emamectin benzoate 1 WG – 0.002%	14.70 (2.68)	9.51^{b} (3.00)	5.26^{b} (2.35)	4.83^{b} (2.13)
T4- Fipronil 5 SC – 0.01%	15.28 (3.30)	3.84^{b} (1.97)	4.22^{b} (2.17)	4.18^{b} (2.13)
T5- Indoxacarb 14.5 SC – 0.015%	8.06 (2.19)	5.75^{b} (2.45)	7.09^{b} (2.75)	5.82^{b} (2.50)
T6-Malathion 50 EC (check) – 0.1%	15.16 (3.30)	7.22^{b} (2.67)	6.67^{b} (2.55)	5.43^{b} (2.32)
T7- Untreated	23.68 (4.52)	23.16^{a} (4.89)	15.35^{a} (4.03)	16.15^{a} (3.73)
CD (0.05)	NS	(1.337)	(0.948)	(0.923)

Mean of five plants
Values shown in parentheses are √x+1 transformed values

Table 4: Effect of new generation insecticides on population of spiders in field conditions

Treatments	Mean population of spiders after third spray*				Mean population of spiders after fourth spray*			
	PTC	**1 DAT**	**3 DAT**	**5DAT**	**PTC**	**1 DAT**	**3 DAT**	**5DAT**
T1- Novaluron 10 EC – 0.015%	0.72^{bc}(1.31)	0.46^{b}(1.21)	0.22^{c}(1.10)	0.22^{bc}(1.10)	0.22^{c}(1.10)	0.22^{bc}(1.10)	0.22^{cd}(1.10)	0.65^{b} (1.29)
T2- Flubendiamide 39.35 SC – 0.0096%	1.23^{a} (1.50)	1.23^{a} (1.50)	1.23^{a} (1.50)	1.47^{a} (1.57)	1.47^{ab}(1.57)	1.47^{a} (1.57)	1.47^{b} (1.57)	2.19^{a} (1.78)
T3- Emamectin benzoate 1 WG – 0.002%	0 .00^{d} (1.0)	0 .00^{c} (1.0)	0 .00^{b} (1.0)	0.22^{bc}(1.10)	0.22^{c} (1.10)	0 .00^{c} (1.0)	0 .00^{d} (1.0)	0.46^{b} (1.21)
T4- Fipronil 5 SC – 0.01%	0 .00^{d} (1.0)	0 .00^{c} (1.0)	0 .00^{b} (1.0)	0.46^{bc}(1.21)	0.46^{c} (1.21)	0 .00^{c} (1.0)	0 .00^{d} (1.0)	0.46^{b} (1.21)
T5- Indoxacarb 14.5 SC – 0.015%	0.46^{c} (1.21)	0.22^{bc}(1.10)	0 .00^{b} (1.0)	0.72^{b} (1.31)	0.72^{bc}(1.31)	0.46^{b} (1.21)	0.46^{c} (1.21)	0.72^{b} (1.31)
T6-Malathion 50 EC (check) – 0.1%	0 .00^{d} (1.0)	0 .00^{c} (1.0)	0 .00^{b} (1.0)	0 .00^{c} (1.0)	0.22^{c} (1.10)	0 .00^{c} (1.0)	0 .00^{d} (1.0)	0.72^{b} (1.31)
T7- Untreated	1.00^{ab}(1.41)	1.23^{a} (1.45)	1.23^{a} (1.50)	1.96^{a} (1.72)	1.96^{a} (1.72)	1.96^{a} (1.72)	2.48^{a} (1.86)	2.74^{a} (1.93)
CD (0.05)	(0.169)	(0.191)	(0.138)	(0.258)	(0.276)	(0.209)	(0.183)	(0.301)

*Mean number of spiders observed in 5 plants
PTC- Pre Treatment Count, DAT- Days After Treatment
Values shown in parentheses are √x+1 transformed values

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