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Dissipation pattern of Emamectin benzoate in/on brinjal fruits

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Abstract
A supervised field trial was conducted to study the dissipation of emamectin benzoate at Naraseepuram village, Thondamuthur, Coimbatore, on the brinjal variety CO 2, during January – March, 2018. The samples were collected up to 15 days after pesticide application along with control and processed by modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method. The final estimation of residues was carried out on Ultra High Performance Liquid Chromatography (UHPLC) with diode array detector. The mean initial deposit after two spraying of emamectin benzoate @ 10 g a.i ha^{-1} and 20 g a.i ha^{-1} in/on brinjal fruit were 0.359 and 0.550 µg g-1, respectively. More than 80 per cent of emamectin benzoate residues got dissipated on 3 days after treatment. The residues persisted up to 3 and 5 days after treatment and further dissipated to Below Detectable Limit (BDL < 0.05 µg g^{-1}) on 5 and 7 days after treatment at @ 10 g a.i ha^{-1} and 20 g a.i ha^{-1}, respectively. Dissipation of emamectin benzoate followed first order reaction kinetics and the calculated half life was 1.53 and 1.57 days, respectively for 10 g a.i ha^{-1} and 20 g a.i ha^{-1}.

Keywords: brinjal, dissipation, emamectin benzoate, half-life, safe waiting period

1. Introduction
Pesticides constitute the key control tactics for management of pests and the productivity of crops depends on their effective control. Together with high-yielding crop varieties and fertilizers, pesticides have helped the Indian farmers in achieving a substantial increase in agricultural productivity (Birthal *et al*., 2000) [5]. The area under plant protection has been continuously increasing in India. The average per hectare consumption of pesticides in Indian agriculture was only 1.2 g ha^{-1} in 1953-54, it increased to 377 g ha^{-1} in 1985-86, 431 g ha^{-1} in 1992 - 93 and further to 600 g ha^{-1} in 2015-16 (Agnihotri, 2000; Anon, 2016). Brinjal (*Solanum melongena* L.) is one of the most popular and economically important vegetables among small-scale farmers and low-income consumers of South Asia and this region accounts for nearly 60 and 53% of world's area and production, respectively. It is grown in almost all states of India with an area of 5.10 lakh hectares under cultivation and production of 88.0 lakh tonnes (Singhal, 2003) [18]. Such an economically important commercial crop is reported variedly to be infested by 142 species of insects, 4 species of mites and 3 species of nematodes (Sohi, 1966) [19], 26 pests (Vevai, 1970) [21], 23 species of insects and 19 diseases (Gowda and Veeresh, 1984) [7], 50 insect pests (Nair, 1995) [12] more than 36 pests (Regupathy *et al*., 1997) [15] from the time of its planting to harvest.

Surveys of farmers have determined that nearly all of the brinjal hectares are sprayed with insecticides to prevent damage from brinjal shoot and fruit borer. Farmers spray insecticides to kill the larva before it enters the fruit (Alam, *et al.* 2003) [2]. The number of sprays on brinjal to control ESFB varies widely from 15 to 40 or more in a single crop season. As a result, farmers produce blemish free fruits, which command premium prices in the market (Dhas and Srivastava, 2010) [6]. Nearly all farmers rely exclusively on application of chemical pesticides to combat insect pests of brinjal. Use of different chemical formulations helped in checking of various insect pests of brinjal but, excessive use of chemical pesticides has destroyed natural enemies, resulting in a resurgence of the pest population. This practice has resulted in tremendous misuse of pesticides, causing a multitude of side effects that includes increased cost of production as well as exposure of farmers and consumers to pesticide residues (Premlata kumara and Basavaraja, 2018) [13]. Their use, however, has limitations and chemical control continuous to be the preferred strategy in practice.

Emamectin benzoate is a novel semi-synthetic derivative of the natural product abamectin in the avermectin family of 16-membered macrocylic lactones. This epi-methyl amino derivative has unprecedented potency against a broad spectrum of lepidopterous pests. Emamectin benzoate is extensively used for the management of shoot and fruit borer in Brinjal. Pesticides are applied to the crops; they may interact with the plant surfaces, be exposed to the environmental factors such as wind and sun and may be washed off during rainfall. The pesticide may be absorbed by the plant surface (waxy cuticle and root surfaces) and enter the plant transport system (systemic) or stay on the surface of the plant (contact). While still on the surface of the crop, the pesticide can undergo volatilization, photolysis chemical and microbial degradation. All these processes can reduce the original pesticides concentration but can also introduce some metabolites in the crops. The products formed may be less or more toxic than the parent chemical. Hence it is important to know the dissipation pattern, half life and waiting period of insecticides applied on the brinjal field to ensure the food safety. With the above background, this study was undertaken to study the dissipation pattern of emamectin benzoate in brinjal.

2. Materials and Methods

A supervised field trial was conducted to study the dissipation of emamectin benzoate at Naraseepuram village, Thondamuthur, Coimbatore, on the brinjal variety CO 2, during January – February, 2018. The experiment was laid out in randomized block design in a plot size of 50 m^2 and replicated thrice, including untreated control. The brinjal plots were sprayed with emamectin benzoate 5 SG - X dose (10 g a.i. ha^{-1}) and 2X dose (20 g a.i. ha^{-1}) twice i.e., first spraying at fruit initiation stage and subsequent spraying at 10 days interval using hand operated knapsack sprayer. It was ensured that the insecticide under investigation had not been used earlier in the experimental plot.

The reference standard of emamectin benzoate (99.4 % purity) was purchased from M/S Sigma Aldrich, Bangalore, India. Acetonitrile of HPLC grade, sodium chloride and anhydrous magnesium sulphate of analytical grade were purchased from Merck (Mumbai, India). Primary Secondary Amine (PSA) (Bondesil 40 µm) and Graphitized Carbon Black (GCB) were purchased from M/S Agilent technologies, USA. Primary stock solutions of emamectin benzoate (400 µg ml^{-1}) standard were prepared with HPLC grade acetonitrile in a volumetric flask. An intermediate stock solution of 100 µg ml^{-1} and 10 µg ml^{-1} was prepared from primary stock solution and working standards was prepared from intermediate stock. All the stock and working standard solutions were stored in the refrigerator at -20 °C.

The linearity study was conducted by injecting five different concentrations of standard solution following three replications of emamectin benzoate. The limit of detection (LOD) was calculated by considering signal-to-noise ratio of three with reference to the background noise obtained from blank sample and the limit of quantification (LOQ) by considering a signal to noise ratio of ten. Recovery studies were conducted to assess the validity of the present method. The homogenized untreated brinjal fruit samples (10g) were spiked at three different concentrations viz., 0.05, 0.25 and 0.5 µg g^{-1} of separately using analytical standard solution of emamectin benzoate. Each treatment was replicated three times with untreated control. The spiked samples were equilibrated for one hour and residues were extracted and estimated as per the method mentioned above. The control brinjal fruit samples were analyzed and the result indicated that blank sample did not contribute any interference with the target compounds. The percentage recovery was calculated by comparing the peak area of the spiked standards with those of the pure standards by using the below formula.

$$Per\ cent\ recovery\ = \frac{Residue\ quantified\ in\ fortified\ level}{Fortified\ level}\ x\ 100$$

One kilogram of fruit sample was collected randomly at 0 (one hour after spraying), 1, 3, 5, 7 and 10 days after spraying from emamectin benzoate treated field along with untreated control separately. The collected samples were transported to the laboratory and processed immediately. The fruits were chopped into small pieces, from which a sub sample of 500 g was taken and homogenized with a mixer grinder. The residues were extracted by following the modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Anastassiades *et al.,* 2003) [3]. A representative sample of 10 g was transferred into a 50 ml centrifuge tube and mixed using a vortexer for one minute after adding 20 ml of acetonitrile. Four gram of anhydrous magnesium sulphate ($MgSO_4$) and 1 g of sodium chloride (NaCl) were subsequently added, shaken well by vortexer and then centrifuged at 6000 rpm for 10 minutes. Nine milli liter of supernatant was transferred to test tube containing anhydrous sodium sulphate (Na_2SO_4) and 6 ml of supernatant aliquot was transferred into a 15 ml centrifuge tube containing 100 mg Primary Secondary Amine (PSA), 600 mg anhydrous magnesium sulphate ($MgSO_4$) and 10 mg Graphitised Carbon Black (GCB). The mixture was vortexed for 1 minute and then centrifuged for 10 minutes at 3000 rpm. The upper extract (4 ml) was transferred into a turbovap tube and concentrated to dryness under a gentle stream of nitrogen in a turbovap LV at 40° C. HPLC grade acetonitrile (1ml) was added to test tube, shaken well reconstituted 1 ml was transferred into a 1.5 ml glass auto sampler vial for analysis. The residues of emamectin benzoate were estimated by Ultra High Performance Liquid Chromatography (UHPLC) (Shimadzu, series 2030) equipped with diode array detector (SPD - M20A), Chromatographic separation was achieved with reverse phase - C18 (Agilent) column, 250 mm length x 4.6 mm id x 5 µ particle size in a column oven, at 40°C. The low pressure gradient condition employed with a mobile phase of acetonitrile and water (60:40) with a flow rate of 1.0 ml $minute^{-1}$ and the injection volume was 20 µl.

The final quantification of pesticide residues was calculated using the following formula

$$Residues\ (ppm) = \frac{As}{Astd}x\frac{Wstd}{Ws}x\frac{Vs}{Asj}$$

Where, As: Peak area of the sample; Astd: Peak area of the standard; Wstd: Weight of the standard in ng; Ws: Weight of the sample in g; Vs: Volume of the sample (final extract in ml); Asj: Aliquot of the sample injected in ml. The insecticide degradation pattern was analyzed by applying seven transformation functions as suggested by Hoskins (1961) [9] and Timme *et al.* (1986) [20]. The half-life was calculated using Pesticide Residue Half Life Calculator software developed by Department of Soil Science, Tamil Nadu Agricultural University, Coimbatore based on Regupathy and Dhamu (2001) [14] and best fit degradation model was determined. The safe waiting period was worked out as per the formula given by Handa *et al.* (1999) [8] using Codex Maximum Residual Limit (MRL)

$$Safe\ waiting\ period\ (T_{tol}) = \frac{\log K_2 - \log(MRL/tolerance)}{\log K_1}$$

Where, K_1 is the slope of the regression line (b), always negative sign used as a positive number and K_2 is the apparent initial deposit obtained by extrapolating the line back to zero time.

3. Results and Discussion

Supervised field trial was conducted in farmers field to study the degradation behavior of emamectin benzoate residues in/on brinjal. Further, fruit samples were collected from insecticides treated plots and subjected to residue analysis in order to study the degradation behavior of the emamectin benzoate. The linearity of the calibration curves was established in the range of 0.05 to 0.8 µg g^{-1} and the correlation coefficient (R^2) obtained was 0.999 (Figure 1). The LOQ and LOD values for emamectin benzoate were 0.015 and 0.05 µg g^{-1} in UHPLC. The results of the recovery study of emamectin benzoate carried out in brinjal fruits (Table 1) revealed that the mean per cent recoveries of emamectin benzoate were 91.35, 89.54 and 92.96 in brinjal fruits with Relative Standard Deviation (RSD) percentage of 3.29, 3.14 and 4.83 when samples were spiked at 0.05, 0.25 and 0.5 µg g^{-1}, respectively. According to SANTE (2017) [16] (Document No. SANTE/11813/2017), any recovery range of 60 to 140 per cent is acceptable for method validation. Based on the recovery study, the suitability of modified QuChERS method for residue analysis of emamectin benzoate in brinjal thus confirmed.

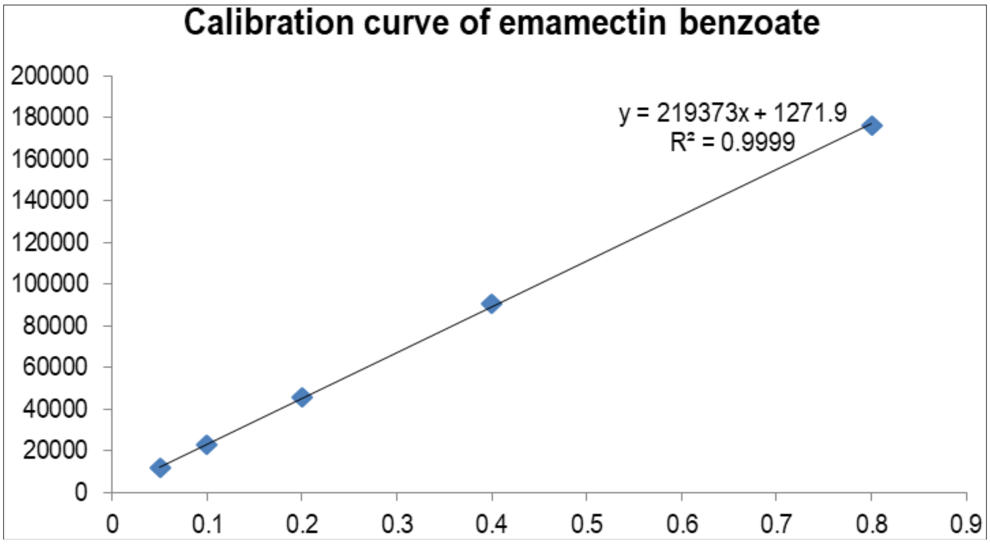

Fig 1: Linearity calibration curve of emamectin benzoate – UHPLC

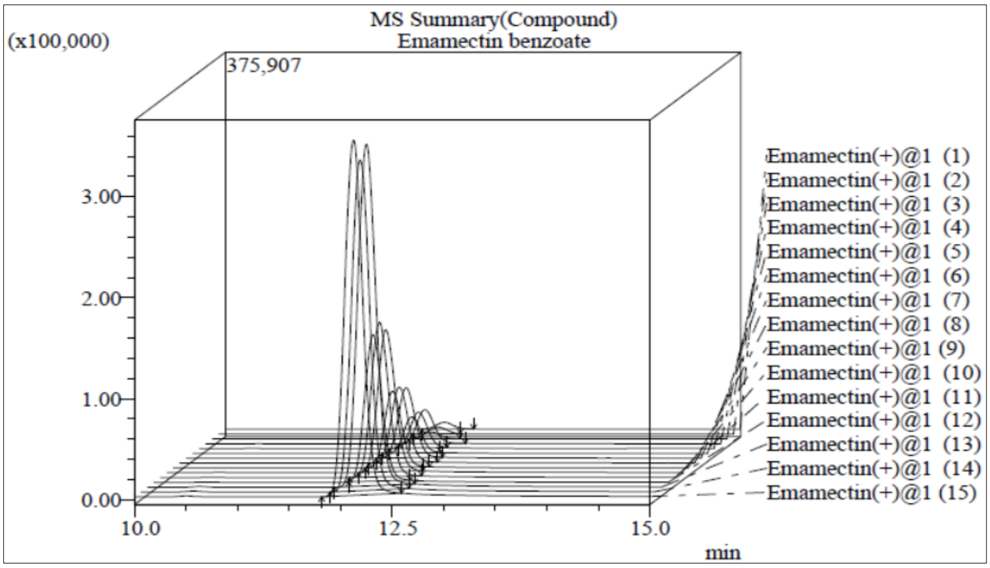

Fig 2: Standard curve of emamectin benzoate – UHPLC

Table 1: Recovery percentage of emamectin benzoate 5 SG in brinjal fruit

Spiked level (µg g^{-1})	Percent recovery (%)			Mean ± SD	RSD
	R1	R2	R3		
0.05	89.20	94.73	90.11	91.35 ± 2.96	3.29
0.25	88.86	87.02	92.73	89.54 ± 2.91	3.14
0.5	93.08	88.18	97.61	92.96 ± 4.72	4.83

Table 2: Persistence and dissipation of emamectin benzoate 5 SG residues in/on brinjal fruit

DAA	Emamectin benzoate 5 SG residues (µg g^{-1})									
	Emamectin benzoate @ 10 g a.i. ha^{-1}					Emamectin benzoate @ 20 g a.i. ha^{-1}				
	R1	R2	R3	Mean	Dissipation (%)	R1	R2	R3	Mean	Dissipation (%)
0 (1 hr)	0.342	0.366	0.370	0.359	0.00	0.540	0.565	0.545	0.550	0.00
1	0.204	0.215	0.209	0.209	41.73	0.315	0.338	0.349	0.334	39.29
3	0.091	0.093	0.088	0.091	74.68	0.123	0.132	0.136	0.130	76.29
5	BDL	BDL	BDL	BDL	100.00	0.050	0.065	0.054	0.056	89.79
7	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	100.00

Table 3: Correlation coefficient and half life for emamectin benzoate in/on brinjal by different methods of transformations of residues data

Function	Emamectin benzoate @ 10 g a.i. ha^{-1}					Emamectin benzoate @ 20 g a.i. ha^{-1}				
	a	b	r	R^2	T Half	a	b	r	R^2	T Half
First order	-1.650	-0.552	-0.999**	0.999	1.53	-0.628	-0.457	-0.999**	0.999	1.57
1.5th order	1.057	0.452	0.996*	0.995	1.23	1.223	0.577	0.994**	0.987	0.87
2nd order	2.449	2.795	0.995*	0.991	0.87	0.409	3.192	0.969*	0.939	0.12
RF First order	-0.877	-0.831	-0.984^{NS}	0.968	0.69	-0.312	-1.061	-0.974*	0.950	0.42
RF 1.5th order	1.454	0.992	0.964^{NS}	0.929	0.25	0.884	1.292	0.934^{NS}	0.873	0.11
RF 2nd order	1.541	4.921	0.940^{NS}	0.884	0.09	-1.187	6.934	0.883^{NS}	0.780	0.03
Inverse power law	-0.064	0.204	0.895^{NS}	0.801	29.69	-0.186	0.301	0.845^{NS}	0.714	9.95

The results of persistence and dissipation of emamectin benzoate in brinjal fruits sprayed at 10 g a.i ha^{-1} and double the recommended dose 20 g a.i ha^{-1} are presented in Table 2. The mean initial deposit (1 hour after spraying) of emamectin benzoate on brinjal fruits was found to be 0.359 and 0.550 µg g^{-1}, at single and double the doses. At recommended dose, emamectin benzoate residue dissipated to 0.209 and 0.091 µg g^{-1} on 1 and 3 days of treatment with dissipation percentage of 41.73 and 74.68 per cent and reached Below Detectable Limit (BDL) of less than 0.05 µg g^{-1} on 5 days after treatment. At double the recommended dose, emamectin benzoate mean residues were 0.334, 0.130 and 0.056 µg g^{-1} with per cent loss of 39.29, 76.29 and 89.79 after 1, 3 and 5 days after spraying, respectively and reached BDL on 7 days after treatment. The dissipation pattern of emamectin benzoate in brinjal fruit was computed following seven transformations and the best fit observed was first order kinetics for both the doses (10 g a.i ha^{-1} and 20 g a.i ha^{-1}) (Table 3). The statistical parameters like intercept (a), slope of regression lines (b) and half life were presented in Table 6. The half life values of emamectin benzoate on brinjal fruit were found to be 1.53 and 1.57 days at recommended and double the recommended dose.

Present study is in accordance with the results of Wang *et al.* (2012) [17, 22], who reported the dissipation half - lives of emamectin benzoate in cabbage, apple and soil were 1.34 - 1.72 day, 2.75 - 3.09 day and 1.89 - 4.89 day, respectively. Minghui Li *et al*., (2011), reported that the half-lives of emamectin benzoate in paddy plants, water and soil were 2.04-8.66 days, 2.89 -4.95 days and 3.65 - 5.78 days with a dissipation rate of 90% over 7 days after application, respectively. The recoveries of emamectin benzoate on cabbage and soil were observed from 71% to 102% at fortification levels of 0.01, 0.1 and 1.0 mg/kg. The reported limit of quantification (LOQ) was found to be 0.01 mg/kg. The dissipation experiments showed the half-lives (T (1/2)) of emamectin benzoate was around 1 days. At pre-harvest intervals (PHI) of 7 and 12 days, emamectin benzoate residue was observed to be below the LOQ (Shuaigang Liu *et al*., 2012) [17]. Emamectin benzoate dissipated rapidly in tea with half-life ($t_{1/2}$) of 1.0 - 1.3 days. The terminal residues of emamectin benzoate were less than 0.062 mg kg^{-1}. The leaching rate of emamectin benzoate from freshly-made tea to brew was <5%. The risk of emamectin benzoate at the recommended dosage was negligible to humans depending on risk quotient (RQ) value, that was lower than 1 significantly (Li Zhou *et al*., 2016) [10]. To conclude, Emamectin benzoate with novel mode of action and high degree of efficacy with short residual toxicity, the half life values of emamectin benzoate on brinjal fruit were found to be 1.53 and 1.57 days at recommended and double the recommended dose, hence it is a very useful tool in IPM programs especially for the management of lepidopterous pests.

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