## **Supplementary Material**

## Action threshold development in cabbage pest management using synthetic and botanical insecticides

Farhan M. Shah<sup>1,2</sup>, Muhammad Razaq<sup>1\*</sup>, Qasim Ali<sup>1,3</sup>, Abid Ali<sup>4</sup>, Sarfraz A. Shad<sup>1</sup>, Muhammad Aslam<sup>5</sup>, Ian C.W. Hardy<sup>2\*</sup>

- <sup>1</sup> Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan 60000, Pakistan
- <sup>2</sup> School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, UK
- <sup>3</sup> Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Kamýcká 1176, Czech Republic

<sup>4</sup> Department of Entomology, University of Agriculture, Faisalabad 38040, Pakistan

<sup>5</sup> COMSATS Institute of Information Technology, Vehari, Pakistan

## \*Corresponding authors:

- Dr Ian CW Hardy, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK. Email: ian.hardy@nottingham.ac.uk. Tel: +44 115 9516052.
- Dr Muhammad Razaq, Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan 60000, Pakistan. Email: muhammadrazaq@bzu.edu.pk. Tel: +92301-7559070

Sowing date	<b>District</b> <sup>2</sup>	Method of sowing <sup>3</sup>	Harvesting date	Insecticide treatment <sup>4</sup>							
				Control	VF-5	VF-10	VF-15	NSE-7	NSE-14	NA-7	NA-14
18/10/2015 <sup>1</sup>	Bahawalpur (B)	Seed in bed	28/04/2016								
10/11/20151	Bahawalpur (B)	Seed in bed	28/04/2016						-		
04/12/20151	Bahawalpur (B)	Seed in bed	24/03/2016						-		
20/01/20161	Multan (BZU)	Transplant to ridge	02/05/2016	•			-		-		
20/02/20161	Multan (KP)	Transplant to ridge	09/05/2016	•				•	-	•	•
15/03/2016	Multan (BS)	Transplant to ridge	02/06/2017	•				•	-	•	•
10/09/2016	Bahawalpur (B)	Seed in bed	11/12/2016	•				•		•	
02/12/2016	Bahawalpur (B)	Seed in bed	13/04/2017	-			•				
02/12/20161	Multan (BZU)	Transplant to ridge	07/03/2017	•				•		•	
23/12/20161	Multan (BZU)	Transplant to ridge	18/03/2017	•				•		•	
25/12/2016	Bahawalpur (B)	Seed in bed	26/04/2017				•			•	
23/01/2017	Multan (BZU)	Transplant to ridge	22/04/2017	•				•		•	
01/02/2017	Multan (BZU)	Transplant to ridge	06/05/2017	•				•		•	
20/02/2017	Multan (KP)	Transplant to ridge	26/05/2017	-			•	•		•	
10/03/2017	Multan (BS)	Transplant to ridge	27/05/2017								

Supplementary Table 1. Overview of agronomic practices and the insecticide treatments employed for trials

In all trails, the cultivar "Charmant" was used for growing cabbage except 15/03/2016 trial, in which cultivar "Golden acre" was used.

<sup>1</sup> Trails were simultaneously infested with lepidopterans and aphids. Therefore to control aphids, imidacloprid was sprayed every 7<sup>th</sup>, 14<sup>th</sup> & 21<sup>st</sup> day in plots that were due to be sprayed with Voliam Flexi (chlorantraniliprole + thiamethoxam) every 5<sup>th</sup>, 10<sup>th</sup> & 15<sup>th</sup> day, respectively.

<sup>2</sup> B= Bindra, BZU= Bahauddin Zakariya University, KP= Kayaanpur, BS= Binda Sindhaila

<sup>3</sup> Beds were planted with two rows of cabbage; ridges were planted with one row

<sup>4</sup> Control= untreated; VF-5= Voliam Flexi sprayed every 5<sup>th</sup> day; VF-10= Voliam Flexi sprayed every 10<sup>th</sup> day; VF-15= Voliam Flexi sprayed every 15<sup>th</sup> day; NSE-7= neem seed extract sprayed every 7<sup>th</sup> day; NSE-14= neem seed extract sprayed every 14<sup>th</sup> day; NA-7= NeemAzal sprayed every 7<sup>th</sup> day; NA-14= NeemAzal sprayed every 14<sup>th</sup> day

 $\blacksquare$  = treatment included in trial,  $\square$  = treatment not included in trial

Year	Sowing date	District*		Lepidopterans							Total
			S. litura	S. exigua	H. armigera	P. xylostella	T. orichalcea	P. brassicae	B. brassicae	M. persicae	
2015-16	18/10/2015	Bahawalpur (B)	-	9	28	210	2	-	7540	-	7789
	10/11/2015	Bahawalpur (B)	-	12	12	128	13	-	13094	173	13433
	04/12/2015	Bahawalpur (B)	-	31	5	110	37	-	798	298	1280
2016-17	10/09/2016	Bahawalpur (B)	632	-	5	-	-	-	-	-	637
	02/12/2016	Bahawalpur (B)	1330	322	3	91	-	-	623	-	2368
	25/12/2016	Bahawalpur (B)	172	14	7	62	-	-	-	-	254
2015-16	20/01/2016	Multan (BZU)	-	-	2	143	-	-	4168	1627	5940
	20/02/2016	Multan (KP)	-	2	6	66	8	-	576	283	940
	15/03/2016	Multan (BS)	511	-	-	98	5	234	-	-	848
2016-17	02/12/2016	Multan (BZU)	-	-	11	58		-	5188	-	5257
	23/12/2016	Multan (BZU)	-	-	15	74		-	4173	-	4262
	23/01/2017	Multan (BZU)	-	-	40	43		-	-	-	84
	01/02/2017	Multan (BZU)	-	-	46	55		-	-	-	102
	20/02/2017	Multan (KP)	489	12	4	23		-	-	-	527
	10/03/2017	Multan (BS)	2302	0	3	28		-	-	-	2333
			<b>Total</b> 5436	402	187	1189	65	234	36160	2381	46054

Supplementary Table 2. Seasonal totals of insect pests from all treatments combined following planting dates

\*Locations within districts: B= Bindra, BZU= Bahauddin Zakariya University, KP= Kayaanpur, BS= Binda Sindhaila

Planting date	Size		Insect	icide		Sample time		Insecticide × Sample time		
	_	F-ratio <sup>1</sup>	df	Р	F-ratio	df	Р	F-ratio	df	Р
10/09/2016	<1cm	5.64	5,12	0.007*	33.59	4,48	< 0.001*	3.76	20,48	0.001*
	1-2cm <sup>†</sup>	14.61	5	0.012*	-	-	-	-	-	-
	$>2cm^{\dagger}$	15.00	5	0.010*	-	-	-	-	-	-
02/12/2016	<1cm	2.26	5,12	0.114*	32.89	3,36	< 0.001*	2.09	15,36	0.095*
	1-2cm	23.54	5,12	< 0.001*	7.84	3,36	0.003*	12.58	15,36	< 0.001*
	$>2cm^{\dagger}$	15.00	5	0.010*	-	-	-	-	-	-
25/12/2016	$<1 \text{cm}^{\dagger}$	10.19	5	0.070*	-	-	-	-	-	-
	1-2cm	2.30	5,12	0.109*	5.58	2,24	0.019*	3.49	10,24	0.014*
	$>2cm^{\dagger}$	14.80	5	0.011*	-	-	-	-	-	-
20/02/2017	<1cm	1.62	5,12	0.228	27.76	3,36	< 0.001*	9.51	15,36	< 0.001*
	1-2cm <sup>†</sup>	12.82	5	0.025*	-	-	-	-	-	-
	$>2cm^{\dagger}$	14.29	5	0.014*	-	-	-	-	-	-
10/03/2017	$<1 \text{cm}^{\dagger}$	10.81	5	0.055*	-	-	-	-	-	-
	1-2cm <sup>†</sup>	14.61	5	0.012*	-	-	-	-	-	-
	$>2cm^{\dagger}$	14.61	5	0.012*	-	-	-	-	-	-

Supplementary Table 3. Effect of insecticide treatment on number of *S. litura* larvae in different size classes

NS indicates non-significant differences (P>0.05).

<sup>1</sup>The test statistic is the F-ratio when both numerator and denominator degrees of freedom are given, otherwise values are the Friedman's test statistic.

<sup>†</sup>Insect count data were treated as detailed in Table 2

\*Significance was adjusted using the FDR procedure. *P*-values remaining significant following this correction are indicated with an asterisk and other results are considered non-significant.

Sowing date	District <sup>*</sup>	Treatment					
		F	df	Р			
18/10/2015	Bahawalpur (B)	25.95	7,16	< 0.001			
10/11/2015	Bahawalpur (B)	45.59	7,16	< 0.001			
04/12/2015	Bahawalpur (B)	41.33	7,16	< 0.001			
010/9/2016	Multan (BZU)	136.18	5,12	< 0.001			
02/12/2016	Multan (KP)	28.53	5,12	< 0.001			
25/12/2016	Multan (BS)	41.77	5,12	< 0.001			
20/01/2016	Bahawalpur (B)	56.45	7,16	< 0.001			
20/02/2016	Bahawalpur (B)	33.45	7,16	< 0.001			
15/03/2016	Multan (BZU)	52.60	7,16	< 0.001			
02/12/2016	Multan (BZU)	195.46	5,12	< 0.001			
23/12/2016	Bahawalpur (B)	69.72	5,12	< 0.001			
23/01/2017	Multan (BZU)	122.95	5,12	< 0.001			
01/02/2017	Multan (BZU)	116.41	5,12	< 0.001			
20/02/2017	Multan (KP)	145.50	5,12	< 0.001			
10/03/2017	Multan (BS)	191.89	5,12	< 0.001			

Supplementary Table 4. Effect of insecticide treatment on percent marketable yield

\*Locations within districts: B= Bindra, BZU= Bahauddin Zakariya University, KP= Kayaanpur, BS= Binda Sindhaila



**Supplementary Figure 1. Seasonal totals of each pest species and their overall profile under different insecticide treatments.** Trials were conducted during the 2015-16 and 2016-17 cabbage growing seasons in the Multan and Bahawalpur districts. Control: no spray; NA: NeemAzal sprayed at weekly (NA-7) or biweekly (NA-14) intervals; NSE: neem seed extract sprayed at weekly (NSE-7) or biweekly (NSE-14) intervals; VF: Voliam Flexi® (chlorantraniliprole + thiamethoxam) sprayed every 5<sup>th</sup> (VF-5), 10<sup>th</sup> (VF-10) or 15<sup>th</sup> day (VF-15). For aphids, imidacloprid (I) was sprayed every 7<sup>th</sup>, 14<sup>th</sup> or 21<sup>st</sup> day in corresponding VF treatments.

500-500-Control 400-I-7 400-I-14 I-21 300-300-NSE-7 NA-7 200-200-100-100-10-Mar 3.Mat 17-Mar 24-Mar 25-100 Mai O-Mai Mai Mar ×en N (c) 2-December 2016, Multan 500-500-400 400 300 300 200 200 100 100-0 0

Sampling date

Supplementary Figure 2. Weekly densities (mean ±SEM) of Brevicoryne brassicae. The pest was present in seven of fifteen trials and four representative examples are illustrated. Control: no spray; NA: NeemAzal weekly spraying (NA-7); NSE: Neem seed extract weekly spraying (NSE-7); (I): Imidacloprid sprayed every 7<sup>th</sup> (I-7), 14<sup>th</sup> (I-14) and 21<sup>st</sup> day (I-21). Arrows indicate dates of peak pest activity in control treatment.

7

1.500

31.721

24-30

10.12

3.30

27.De

20.0

3.30

10.33

Mean number of aphids per plant



(a) 18-October 2015, Bahawalpur

7.500

31.300

24-125

7-325



(b) 10-November 2015, Bahawalpur



Sampling date

**Supplementary Figure 3. Weekly densities (mean ±SEM) of** *Plutella xylostella.* The pest was present in fourteen of fifteen trials and six representative examples are illustrated. Control: no spray; NA: NeemAzal weekly spraying (NA-7); NSE: Neem seed extract weekly spraying (NSE-7); VF: Voliam Flexi (chlorantraniliprole + thiamethoxam) sprayed every 5<sup>th</sup> (VF-5), 10<sup>th</sup> (VF-10) and 15<sup>th</sup> day (VF-15). Arrows indicate dates of peak pest activity in control treatment.



**Supplementary Figure 4. Weekly densities (mean ±SEM) of** *Helicoverpa armigera*. The pest was present in twelve of fifteen trials and six representative examples are illustrated. Treatments are as defined in Supplementary Fig. 3. Arrows indicate dates of peak pest activity in control treatment.



Insecticide treatment

**Supplementary Figure 5. Effect of insecticides on seasonal totals of each larval size class of** *Helicoverpa armigera* (small: <1cm; medium: 1-2cm; large: >2cm). The pest was present in twelve of fifteen trials and six representative examples are illustrated. Treatments are as defined in Supplementary Fig. 3.



**Supplementary Figure 6. Weekly densities (mean ±SEM) of** *Spodoptera litura* (combined across all larval size classes) under different insecticide treatments for given planting dates. The pest was present in six of fifteen trials and four representative examples are illustrated. Treatments are as defined in Supplementary Figure 3.



**Supplementary Figure 7. Weekly densities (mean ±SEM) of** *Spodoptera litura* by larval size class. The pest was present in six of fifteen trials and three representative examples are illustrated. Treatments are as defined in Supplementary Fig. 3. Arrows indicate dates of peak pest activity in control treatment.



(b) 10-September 2016, Multan

(c) 2-December 2016, Bahawalpur



Supplementary Figure 8. Mean ( $\pm$ SEM) seasonal totals of *Spodoptera litura* egg batches under different insecticide treatments for given planting dates. Treatments are as defined in Supplementary Figure 3. Bars with common letters do not differ significantly (P>0.05; Tukey HSD test).