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

Short title: *Action thresholds: synthetics and botanicals*

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Abstract

As synthetic insecticides can have environmentally detrimental side effects, it is desirable to limit their use while still achieving good marketable yield. One approach is to apply pesticides only when needed, as determined by an action threshold (AT), defined as the number of pests per crop plant or damage intensity at which application is recommended. Another approach is to adopt alternative pesticides, which can also be applied according to ATs. Here, ATs are developed in cabbage pest management using both approaches against the moths *Plutella xylostella* (L.), *Helicoverpa armigera* (Hübner) and *Spodoptera litura* (F.) and the aphid *Brevicoryne brassicae* (L.). Action thresholds were derived using fixed spraying regimes for the synthetic insecticides (imidacloprid and Voliam Flexi) and for azadirachtin, a neem-derived botanical. Botanical insecticide was as effective as synthetics in suppressing pests and protecting yield. For synthetics, derived ATs are 40 individuals per plant for *B. brassicae*, 0.3 larvae for *P. xylostella* and 0.2 medium-sized larvae for *H. armigera* and for *S. litura*. For *H. armigera* and *S. litura*, negative relationships between marketable yield and pest were found when larvae were medium or large sized, but not when larvae were small. Compared to synthetics, benefits of using neem formulations include higher action thresholds against *P. xylostella* (0.6/plant) and *H. armigera* (0.4/plant) and an oviposition deterrent effect against *S. litura*. Although regional limits may apply to the accuracy of any ATs derived, the approach used towards their establishment is simple and transferable to other agricultural regions and crops.

Key words: Insect pests, losses, marketability, larval phenology, oviposition, azadirachtin

Introduction

The general reliance, by growers, on pesticides to control pests has been generated by their perceived effectivity and by their simplicity of application (Leach et al. 2017). While the contribution of synthetic chemical pesticides to yield enhancement is undeniable, their injudicious use has led to adverse effects on non-target organisms (Desneux et al. 2007), selected for pest resistance (Bass et al. 2015) and polluted environments (Singh et al. 2018). Integrated pest management (IPM) provides growers with a relatively simple decision making tool, the action threshold (AT), which justifies treatment when the pest or their level of damage to economic value of crop exceeds tolerable levels (Shah et al. 2019), rather than applying pesticide on a calendar (fixed time) basis without pest evaluation (Badenes-Perez & Shelton 2006; Weinberger & Srinivasan 2009). ATs represent a quantifiable relationship between the pest density and their potential ability to cause yield loss. ATs can be developed using both a research-based approach or prior experience of the crop-pest relationship (Nault & Shelton 2010), do not necessarily require complex models (Nault & Shelton 2010) and can be adjusted for varieties, environmental conditions (Nault & Shelton 2010), biocontrol services (Walker et al. 2010) and according to local economic and market conditions (Shah et al. 2019).

Another approach for reducing applications of synthetic pesticides is to develop non-synthetic alternatives, ‘botanicals’, obtained from plants (Stevenson et al. 2017; Leather & Pope 2019; Maazoun et al. 2019). The scope of interest in botanicals is not only confined to agriculture use (Fekri et al. 2016) but also to insect pests of veterinary and medical importance (Krčmar & Gvozdić 2016) and urban environments (Bacci et al. 2015). Interest in botanicals is growing due to several favourable properties, such as low human toxicity, easy degradation and environmental safety (Isman 2008; Isman & Grieneisen 2014). While the use of botanicals has been thought to be generally less harmful to non-target organisms than are synthetics (Gahukar 2000; Charleston et al. 2006) they can still cause adverse effects on the physiology and behavior of pollinators (Christen et al. 2018) and biocontrol agents (Monsreal-Ceballos et al. 2018) and the inclusion of botanicals into pest management programes should thus proceed with caution. Development of ATs for these, as well as for synthetics, is thus desirable. The suitable use of botanicals could be especially valuable in developing countries (Amoabeng et al. 2013; Amoabeng et al. 2014) where the source plant species are often locally abundant and accessible and the preparation of extracts is inexpensive (Boursier et al. 2011; Isman 2014). One such plant of interest is the neem tree (*Azadirachta indica*, A. Juss. (L.), Family: Meliaceae), which is native to the Indian-subcontinent and grown in at least 30 countries in Asia, Africa and the Americas (Kumar & Navaratnam 2013). Neem trees are a source of azadirachtin, a major active ingredient (Pascoli et al. 2019) known to adversely affect the biological performance of a wide range of target pests (Mordue & Blackwell 1993). Although azadirachtin has been effectively trialed in agricultural pest control across a range of cropping systems (Shah et al. 2017; Shah et al. 2019) the adoption of neem-derived botanicals should be cautious. One study has found that azadirachtin is equally toxic to bees as the synthetic neonicotinoid imidacloprid (Bernardes et al. 2017). Moreover, direct or indirect exposure of egg parasitioid, *Trichogramma chilonis* Ishii individuals to azadiracthin reduced their survival (Raguraman & Singh 1999). Further, azadirachtin formulations have little residual in-field stability, necessitating intense exposure at short intervals (Dhingra et al. 2008; Shah et al. 2017) which can impair beneficial ecosystem services provided by pollinators and parasitoids.

Here action thresholds are developed, for both synthetic insecticides and neem-derived botanicals, against insect pests of cabbage, *Brassica oleraceae* var. *capitata*, a high-value cash crop grown worldwide. Cabbage is fed upon by numerous lepidopteran and aphid species. Prominent pests among these are the moths *Plutella xylostella* (L.) (Lepidoptera:

Plutellidae), *Helicoverpa armigera* (Hübner) and *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) and the aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). Aphids cause yield losses by sucking phloem sap or by producing honey dew, which affects photosynthesis, and also by vectoring pathogens (Pallett et al. 2002). By feeding on leaves, lepidopterans reduce photosynthetic ability and also reduce the market value of harvested produce via cosmetic injury.

Synthetic pesticides are the currently the most widely adopted crop protection practice among many cabbage growers (Mazlan & Mumford 2005; Reddy 2011). ATs were developed for spraying synthetics against *P. xylostella* (Reddy & Guerrero 2001), but there are almost no ATs established for using synthetics to combat infestation in cabbage by *S. litura*, *H. armigera* or *B. brassicae*. Similarly, although botanicals, derived from several native plant species, have been used against cabbage pests with economically good results in Africa (Amoabeng et al. 2013; Amoabeng et al. 2014) and the use of neem against cauliflower (Shah et al. 2019) and tomato pests (Reddy & Tangtrakulwanich 2013) has been encouragingly trialed in the Indian sub-continent and elsewhere, there has been no development of ATs for applying botanical pesticides to cabbage crops. In common with a companion study on cauliflower crops (Shah et al. 2019), this study was aimed at deriving such ATs for the application of neem-derived azadirachtin to cabbages and also for the more commonly applied synthetic insecticides. The approach for deriving ATs involved spraying insecticides at pre-determined intervals on crops sown at different dates, observing pest species, numbers and phenology, and taking into account both yield and the marketability of the harvested crop.

Materials and methods

Study area

A series of field experiments was conducted mainly in well-managed fields belonging to commercial farmers in Multan (Moza Kayaanpur 30°12'78.0"N, 71°45'58.5"E and Moza Binda Sindhaila 30°14'05.7"N, 71°24'47.4"E) and Bahawalpur districts (Moza Bindra 29°41'93.2"N, 71°64'73.4"E) in the Punjab province of Pakistan. A small number of experiments were conducted at the Agriculture Research Farm of Bahauddin Zakariya University, Multan (BZU) (30°25'70.5"N, 71°51'22.1"E). Both Bahawalpur and Multan districts have typically hot and dry climates. Bahawalpur, around 100km to the south of Multan, is close to the Cholistan desert.

Field experiments

Fifteen experiments were conducted during two cabbage growing seasons, 2015-16 and 2016-17. Sowing was between mid-September and late-December for the six experiments in Bahawalpur district (Fig. 1c) and between early-December and mid-March for the nine experiments in Multan district (Fig. 1c). In Multan district, all cabbages were grown from nursery-prepared 4-5 week old plants, transplanted onto single sided ridges (100cm apart). In Bahawalpur district, cabbage seeds were sown directly into double sided beds (60cm wide) using manual dibbling (3 seeds per dibble, thinned to one plant after seedling germination). In all cases, seedlings were spaced 30cm apart along each row. Nearby plots were separated by 1.5m buffer zones to avoid spillover effects.

Experimental cabbage fields were divided into blocks with treatment plots (three replicates of each type) allocated among blocks using a randomized complete block design. Individual treatment plots comprised four 6m-long rows. Fields were visited twice per week until pests were first observed; thereafter the methods below were followed.

Insecticides

Synthetic insecticides and neem-derived botanical compounds were used. The synthetics were the neonicotinoid imidacloprid (I; Confidor®, 20% SL, Bayer Crop Science, Pvt. Ltd.), and Voliam Flexi® (VF; a mix of chlorantraniliprole [an anthranilic diamide] and thiamethoxam [a neonicotinoid], Syngenta Crop Science, Pvt. Ltd., Karachi, Pakistan). Botanicals were the commercial oil formulation NeemAzal T/S® [NA] (azadirachtin-A, 10g/L, Trifolio GmbH, Germany) and a self-prepared neem seed extract (NSE). For preparing NSE, seeds were crushed into powder-form using electric blender (Moulinex®, model A276). Around 100g of ground seeds were tied in a muslin cloth and soaked in one liter of water for seven days, following the method of Boursier *et al.* (2011). The resultant extract is a rich source of azadirachtin A, with a concentration of around 200 mg in one liter water (Boursier *et al.* 2011).

Manufacturer-recommended doses for Voliam Flexi (active ingredient, AI, 51.96g/ha) and imidacloprid (AI 98.8ml/ha) were used. Voliam Flexi and imidacloprid were mixed in water at 0.17g/L and 0.33ml/L water, respectively. NeemAzal was mixed in water at 1.2ml/L water. NSE was diluted to a 5% aqueous solution (50ml/L) before application.

All insecticides were applied as foliar sprays using a hand operated knapsack sprayer (PB-20; Cross Mark Sprayers, Johor, West Malaysia) fitted with a hollow cone nozzle. Separate sprayer tanks were used for botanical and synthetic insecticides. The water volume used for spraying a treatment plot ranged between five to seven liters, depending upon the growth stage of the crop.

Experimental treatments

All insecticides were sprayed at predetermined intervals (Supplementary Table 1). Voliam Flexi and imidacloprid were the synthetic insecticides used against lepidopteran and aphid pests, respectively. These insecticides were sprayed at different time intervals (treatments) (Supplementary Table 1). When experimental cabbages were infested with lepidopterans only, Voliam Flexi was sprayed every 5th, 10th or 15th day; and when only aphids and no lepidopterans were present, plots that were due to be sprayed with Voliam Flexi were instead sprayed with imidacloprid every 7th, 14th or 21st day. However, when lepidopterans and aphids infested cabbages simultaneously, three plots sprayed with Voliam Flexi were also sprayed with imidacloprid. The shortest interval by either insecticide represents the normal practice of the local growers. Further experimental treatments were: spraying botanical NeemAzal or NSE at weekly intervals (both were collectively used for aphids and/or lepidopteran pests and the inclusion of control plots. This was the core protocol employed during both study years. For both neem-derived botanicals, fortnightly spray regimes in the first year were also trialed but, as these transpired to be less effective than their weekly-applied counterparts (see Results), they were not included in the second year of trials.

Sampling and yield assessment

Pest sampling was carried out on a weekly basis between initial pest appearance and the time of crop harvest. Incidence of attack by lepidopteran larvae and aphids was measured as number of insect pests on 10 randomly selected plants per replicate per treatment (Reddy 2011; Amoabeng et al. 2013). For the 2015-16 cabbage growing season, larvae were recorded without reference to their size. In the following year, the size classes of *H. armigera* and *Spodoptera* spp. larvae were also noted (small <1cm, medium 1-2cm or large >2cm in length) (Shah et al. 2019) and also the egg batches laid by *Spodoptera* spp. were counted (Shah et al. 2019). Due to their small size, *P. xylostella* were recorded in terms of numbers only (Burkness & Hutchison 2008). Pest specimens were deposited in the IPM laboratory at the Department of Entomology, BZU, Multan, Pakistan.

On crop maturity (when 80-90% heads attained marketable size), 100 cabbage heads per treatment were harvested and evaluated following a 1-6 damage rating scale (Greene et al. 1969) for assessing marketable yield of harvest. Cabbage heads scoring 1-3 were considered marketable. Injured heads by cabbage borers (*S. litura*, *S. exigua* and *H. armigera*) were opened to obtain information on pest species present whereas *P. xylostella* and *B. brassicae* were easily observed within head leaves. For deriving action thresholds, the acceptably marektability criterion was set at 90%, following local grower practice for commercial sale.

Statistical analyses

For each site in a particular year, all trials were used for the assessment of the effects of planting date and insecticide treatment on pests. Effects on seasonal totals (weekly records per plant summed across sampling dates) of each pest species were assessed using analyses of covariance (ANCOVAs). Because several ANCOVA tests were carried within years and sites (i.e. for each pest species), possible Type I errors were controlled for using the false discovery rate (FDR) procedure with the family-wide α-value set to 0.20 (Benjamini & Hochberg 1995; McDonald 2014). Effects on species composition (the profile of the guild of pest species) were assessed using multivariate analysis of variance (MANOVA), fitting insecticide a factor and planting date as a covariate. Effects of insecticides on the *S. litura* oviposition (assessed as the seasonal total number of egg batches per plant) were evaluated using ANOVA, followed by Tukey's HSD test, for each planting date.

Weekly effects of insecticides on abundance of each species present were assessed using repeated measures ANOVA with insecticides and sampling dates fitted as factors. For *S. litura*, the numbers of small, medium or large larvae were analyzed separately using repeated measures ANOVA. Count data were X+1 $\log_{10}$ transformed, and sampling dates with zero insects present were excluded to improve compliance with the standard assumptions of normally distributed errors with homogeneous variances. If transformed data did not meet these assumptions, insecticide effects on seasonal totals of the pest species were assessed using non-parametric Friedman's tests. Because several tests of the effects of insecticide, sample time and their interaction were carried out on each species, the significance criterion was adjusted using the FDR procedure (Benjamini & Hochberg 1995; McDonald 2014).

Percent marketable yield obtained from insecticide treatments were arcsine-square root transformed prior to ANOVA. Relationships between pests (total numbers or larval size class) and marketable yield were assessed using regression analysis. All data analyses were performed using the SPSS software package (version 21).

Results

The guild of pest insects associated with cabbage comprised two aphid and six lepidopteran species (Fig. 1a,b). In terms of abundance and persistence, the dominant lepidopterans were *S. litura*, *P. xylostella* and *H. armigera,* and the dominant aphid was the apterous form of *B. brassicae* (Fig. 1a,b). *Spodoptera litura* and *H. armigera* constituted the most persistent pest complex, present mostly concurrently in October and November and again from February to early-June (Fig. 1a). The pest complex was diversified by the appearance of *P. xylostella* and *B. brassicae* from early-January and infestation continued until April or May (Fig. 1a,b). Remaining pests (*T. orichalcea*, *P. brassicae*, *S. exigua* and *M. persicae*) were infrequently present and at lower densities and were thus considered as minor pests (Fig. 1a,b). As minor pests can be managed as an indirect consequence of the management employed for major pests, major pests were the focus for presenting results and deriving ATs.

Effect of planting date and insecticide treatment on overall pest numbers

The overall composition of pest (species and numbers) present was influenced by the date of planting (Fig. 1c) as well as by insecticide treatment (MANOVAs; Table 1; Supplementary

Fig. 1). Pest densities were typically abundant in untreated plots within each site in each year (Supplementary Fig. 1). Spraying plots with insecticide (whether synthetic or botanical) suppressed pest numbers. NeemAzal resulted in better pest suppression than neem seed extract. Weekly spraying of either neem formulation suppressed pests better than fortnightly spraying (Supplementary Fig. 1a,c).

For individual pest species, effects of planting date and insecticide treatment were usually significant in the case of *B. brassicae*, *H. armigera*, *P. xylostella* and *S. litura* (with all sizes combined) and the minor pests (ANCOVAs; Table 1; Supplementary Fig. 1). *Brevicoryne brassicae*, *P. xylostella* and *H. armigera* were abundant when cabbages were grown between October and January whereas *S. litura* was abundant when cabbages were grown either in September or from February to March (Fig. 1c).

Effect of insecticides on weekly abundance of pest insects

The effect of insecticides, sampling dates and their interactions were typically significant for *B. brassicae*, *S. litura*, *P. xylostella* and *H. armigera*, and also for minor pests (Table 2). *Brevicoryne brassicae* was present in eight out of 15 trials, whereas *S. litura* was present in six of these. However, *H. armigera* and *P. xylostella*, were found in almost all trials (14/15; Table 2). Patterns of weekly abundance of each of these four pests are illustrated in Supplementary Figures 2-5.

Among insecticide treatments, spraying Voliam Flexi every 5th day (against lepidopterans) and/or imidacloprid (against aphids) every week, suppressed pests to the lowest numbers observed (below 0.30, 0.2 and 20 individuals per plant for *P. xylostella, H. armigera* and *B. brassicae*, respectively). Weekly spraying with NeemAzal suppressed these pests to below 0.6, 0.4 and 40 individuals per plant, respectively (Supplementary Figs. 2-4). Although insecticide spraying in other plots was usually equally effective, on some sampling dates higher pest densities were noted. On these dates, in plots with Voliam Flexi sprayed every 10th day and/or imidacloprid sprayed every 14th day, densities of *P. xylostella, H. armigera* and *B. brassicae* reached 0.6, 0.6 and 40 individuals per plant, respectively (Supplementary Figs. 2, 3d,e, 4a,b) whereas in plots with Voliam Flexi sprayed every 15th day, imidacloprid sprayed every 3rd week or weekly sprays of NSE, densities reached 1, 0.6 and 50 individuals, respectively or even higher in case of NSE for some planting dates (Supplementary Figs. 2, 3d,e, 4a,b).

While mean per plant densities of *H. armigera* were usually similar among insecticide treatments (Voliam Flexi and NeemAzal), there were differences in the sizes of pest larvae present. When plots were sprayed with Voliam Flexi every 5th day or weekly with NeemAzal, larvae were typically small or medium sized, with large larvae seldom observed. In contrast, medium and large larvae were abundant when plots were sprayed with other insecticide treatments (Supplementary Fig. 5).

For *S. litura*, seasonal trend of pest suppression across treatments was unclear when the total numbers (all larval sizes combined) were considered (Supplementary Fig. 6). Considering pest by size class (Burkness & Hutchison 2008; Shah *et al.* 2019) found that effects of insecticides were typically significant for each class (Supplementary Table 3). Small larvae were present in higher numbers, and there was no consistent pattern of pest suppression across treatments, thus action thresholds could not be identified for small larvae (Supplementary Fig. 7a,d,g). Medium larvae were typically supressed below 0.2 per plant in plots sprayed every 5th or 10th day with Voliam Flexi or weekly with NeemAzal. There was seldom any such pest suppression when plots were sprayed with Voliam Flexi every 15th day or weekly with NSE (Supplementary Fig. 7b,e,h). Large larvae were rare after spraying with Voliam Flexi every 5th day or NeemAzal. However, higher densities of large larvae were found (*ca*. 0.2 larvae per plant) in other insecticide treated plots (Supplementary Fig. 7c,f,i).

Effect of insecticides on abundance of **S. litura** *egg batches*

Spodoptera litura egg batches were found in six of the fifteen trials. Egg batch abundance was significantly affected by insecticide treatments in five of these trials (2015-16 sowing date: March 15th: $F_{5,12}=28.24$, $P<0.001$. 2016-17 sowing dates: December 2nd: $F_{5,12}=19.33$, $P<0.01$; December 25th: $F_{5,12}=18.75$, $P<0.01$; February 20th: $F_{5,12}=7.07$, $P=0.003$; March 10th: $F_{5,12}=67.17$, $P<0.001$). When treatment affected abundance, egg batches laid were typically more abundant in plots sprayed with Voliam Flexi every 5th day (Supplementary Fig. 8) while abundance was lower in plots sprayed weekly with neem formulations.

Marketability

In all trials, the percentage of marketable yield was significantly affected by insecticide treatment ($P<0.001$ in all cases: Supplementary Table 4). In untreated plots, loss to marketable yield by *B. brassicae*, *H. armigera*, *P. xylostella* and *S. litura* reached 44%, 40%, 35% and 98%, respectively (Fig. 2). In a few trials, *S. exigua* larvae were recovered from infested heads but their proportional loss was not more than 10% in untreated plots (Fig. 2). Although spraying synthetics and neem-derived compounds protected yield losses, the acceptable criterion for marketability (set at 90% of yield) was only achieved when plots were sprayed with Voliam Flexi every 5th day or when sprayed weekly with NeemAzal (Fig. 2). Spraying Voliam Flexi every 10th day only sometimes produced yields that were at least 90% marketable (Fig. 2).

Predictors of marketable yield

The identification of predictors of yield is fundamental to the derivation of ATs. The companion study on cauliflower crops found that peak pest infestation, across an extended period of infestation, could be used as a reliable predictor for yield (Shah et al. 2019), and approach was followed here. Weekly pest records for each species were converted to cumulative insect days, a crop protection index which summarizes infestation records in terms of magnitude and duration (Ruppel 1983; Shah et al. 2019). Cumulative insect days for *B. brassicae*, *H. armigera*, *P. xylostella* and *S. litura* were calculated by subtracting the mean density per plant of each pest at the current evaluation date from the mean observed at the previous evaluation date, and multiplying that difference by the days between evaluations and lastly by summing these calculations (Ruppel 1983). There were strong correlations between the peak infestations and the cumulative insect days for all four species (Regressions: *B. brassicae*, $F_{1,10}$= 165.69; P<0.001; r^2= 0.94; *H. armigera*, $F_{1,40}$= 362.93; P<0.001; r^2= 0.96; *P. xylostella*, $F_{1,40}$= 194.10; P<0.001; r^2= 0.89; *S. litura*, $F_{1,16}$= 47.68; P<0.001; r^2= 0.74; Fig. 3) and then assessed relationships between peak infestations and yield. Peak infestation and marketable yield were significantly correlated for *B. brassicae* ($F_{1,21}$= 257.40; P<0.001; r^2= 0.77; Fig. 4a) and *P. xylostella* ($F_{1,68}$= 257.52; P<0.001; r^2= 0.69; Fig. 4c). For the two lepidopterans that were recorded by size class, the relationship was significant when larvae present were of medium (*H. armigera*: $F_{1,38}$= 86.43; P<0.001; r^2= 0.84; *S. litura*: $F_{1,28}$= 217.27; P<0.001; r^2= 0.88) or large size (*H. armigera*: $F_{1,38}$= 93.97; P<0.001; r^2= 0.77; *S. litura*: $F_{1,28}$= 254.14; P<0.001; r^2= 0.89; Fig. 4b,d) but not when the larvae were small (*H. armigera*: $F_{1,38}$= 2.48; P=0.123; r^2= 0.061; *S. litura*: $F_{1,28}$= 0.38; P=0.543; r^2= 0.013).

Action thresholds

Peak pest density was used to identify action thresholds (Hines & Hutchison 2001; Saeed et al. 2018): peak pest density was used from those insecticide treatments that could attain high yield (>90%), while treatments that could not attain high yield were considered ineffective in protecting yield losses and less important for identifying action thresholds.

The above information allows recommendation of the following action thresholds that should result in at least 90% marketable cabbage yield. If applying the synthetic insecticides trialed, crops should be sprayed with Voliam Flexi when densities reach an average of 0.3 larvae per plant for *P. xylostella*, irrespective of the size of the larvae, and 0.2 medium-sized larvae for both *H. armigera* and *S. litura*. For the aphid *B. brassicae*, the recommended action threshold for spraying with imidacloprid is 20-40 individuals per plant. A range, rather than a number, is given as aphids have great potential for rapid clonal multiplication during the parthenogenetic phases of their life-cycles (Foster 2002).

If applying the neem-derived botanical insecticides that have been trialed, cabbage crops should be sprayed with NeemAzal at densities of 0.6 larvae per plant for *P. xylostella* and the recommended action threshold is 0.2 medium-sized larvae for *S. litura.* For *H. armigera*, the action threshold is 0.2 to 0.4 medium-sized larvae: a range is given because most trials suggest a value of 0.2 but in several trials densities of 0.4 larvae per plant did not prevent marketable yield from attaining 90%. For *B. brassicae* the recommended action threshold is 40 individuals per plant.

Discussion

Cabbages in all trials were infested with a complex of pests, comprising lepidopterans (September to November and April to early-June) and aphids (late December to early-April). Of the eight pests species observed, four (*P. xylostella, H. armigera, S. litura and B. brassicae*) were abundant, causing substantial yield losses, and are thus deemed major pests. Within the pests' activity periods, crops sown between the months of October and January harbored more *P. xylostella, H. armigera* and *B. brassicae,* whereas crops sown between February and March had greater infestations of *S. litura*. This accords with prior studies which have found that planting date affects the phenological association between host plants and their pest herbivores (Siddiqui et al. 2009; Vanlaldiki et al. 2013). Adapting planting dates as a pest management strategy is likely to influence both the relative importance of species within the complex of pest herbivores (Saeed et al. 2015) and, in consequence, the optimal insecticide application program. In the case of vegetable production, market prices can vary on a monthly basis, depending upon consumer demand (generally higher for the first and last crops of a season). Thus, planting date adjustment is unlikely to be adopted by commercial scale growers but may be used by subsistence growers and also serves to identify periods when pests are likely to become abundant and thus the frequency of control required.

The major pests in the current study have been identified as a significant threat to cruciferous crops in many countries (Yankanchi & Patil 2009; Reddy 2011; Labou et al. 2017; Shah et al. 2019) and insecticides, due to their rapid action, have been the most adopted control measure among growers, despite increasing realization of their undesired effects. IPM considers strategies that can limit or replace excessive reliance on pesticides in order to diminish negative effects while maintaining or improving pest control. Using action thresholds and exploring alternative pesticides are two key components of this. A common method of identifying action thresholds is to decide upon and trial some candidate values, and subsequently adopt those that perform best (Reddy & Guerrero 2001). However, without some prior information with which to choose values to trial, this approach risks not including the ideal AT within the range trialed. In the present case, such prior information was lacking (i.e. ATs have not been reported previously for many cabbage pests) and thus a variety of fixed interval spraying regimes was used to obtain a number of pest infestation ranges. These obtained ranges were used to generate information on how different levels of pest infestation relate to marketable yields, and thus identify the associated action thresholds.

Voliam Flexi (chlorantraniliprole + thiamethoxam) and imidacloprid were used in trials as these are the synthetic insecticides most widely used against aphids (Razaq et al. 2011; Shah et al. 2017) and lepidopterans (Liu et al. 2017), respectively. Imidacloprid acts selectively on the insect nicotinic acetylcholine receptor (Jeschke & Nauen 2008) and chlorantraniliprole in

Voliam Flexi acts by selectively binding to ryanodine receptors in muscle cells, resulting in the uncontrolled release of calcium stores (Lahm et al. 2005); both may be relatively non-toxic to beneficial biocontrol agents (Karthik et al. 2015; Liu et al. 2016) but there is also ongoing concern regarding effects of neonicotinoids on insect pollinators (Godfray et al. 2015; Jactel et al. 2019). Azadirachtin was used as it is an important botanical insecticide, is available in commercial oil formulations and as seed aqueous extracts, and known to affect both aphid and lepidopteran pests (Razaq *et al.* 2011; Reddy 2011; Shah *et al.* 2017; Shah *et al.* 2019). Although it is registered for commercial use in many countries (Kleeberg 2004; Kleeberg et al. 2010) its application should be cautious due to undesired effects on non-target organisms (Gontijo et al. 2015).

If was found that more frequent spraying with synthetics always resulted in high yield (>90%) and that less frequent spraying did not, although at most times pest densities were similar (as observed for *P. xylostella* and *B. brassicae*). While similar results were obtained for *H. armigera* and *S. litura*, there were clear differences in terms of larval phenology: more frequent spraying killed pests when they were small and less frequent spraying (every 10th or 15th day) allowed pests the opportunity to feed and grow to sizes that can cause rapid damage (Smits et al. 1987; Wightman et al. 1995; Cherry et al. 2000; Liburd et al. 2000). Similar to conclusions of the companion study on cauliflower (Shah et al. 2019), the findings of the current study suggest that small *H. armigera* and *S. litura* larvae have no discernable effect on marketable yield and thus that only the numbers of medium and large sized larvae, which negatively affect yield, should be used in the action threshold decision. Consideration of larval phenology is thus likely to reduce the intensity of control measures applied compared to under fixed spraying schedules (Mazlan & Mumford 2005; Reddy 2011), resulting in better economic returns.

Spodoptera litura laid the most egg batches in plots sprayed with Voliam Flexi every 5th day and the least in plots sprayed with neem formulations. As a preference for damage-free host plants for oviposition is known in other species of *Spodoptera* (Zakir et al. 2013), data from the current study suggest that while frequent spraying with Voliam Flexi killed any small larvae present, it also resulted in cabbage plants being more attractive to adult female *S. litura* seeking egg laying sites. Spraying with NeemAzal similarly killed small larvae but subsequent oviposition was less common than under Voliam Flexi treatment, suggesting that azadirachtin acted as an oviposition deterrent (see also (Kleeberg et al. 2010)). Action thresholds derived from NeemAzal were higher than those derived from synthetics. This can result from the increased pest control efficiency due to a diverse array of effects on target pests: azadirachtin based insecticides can act as antifeedants, sterilents, growth inhibitors and toxicological repellents (Verkerk & Wright 1993; Gahukar 2000; Ahmad et al. 2013; Ahmad et al. 2015), keeping pests under physiological stress and increasing their susceptibility to natural enemies (Charleston et al. 2006).

In the trials reported here, spraying with neem seed extract led to considerably greater yields than in unsprayed plots and, in accord with prior studies (Shah et al. 2017; Shah et al. 2019), was as effective as NeemAzal in suppressing oviposition by the major pest *S. litura*. However, action thresholds for its application could not be derived as it was frequently unable to protect plants sufficiently to produce >90% yield. Despite its moderate effect in terms of pest suppression, NSE is relatively inexpensive (Shah et al. 2019) and could be an asset when crops are grown for subsistence. However, for crops grown at a commercial scale, any potential for NSE as an alternative pesticide will most likely be realized via its integration with other control measures.

In conclusion, cabbage is attacked by an array of pests which can be suppressed by insecticides. Action thresholds (ATs) have been derived and recommended for spraying synthetic and botanical insecticides against the most abundant of these pests and it has been shown that in some cases they should be attuned to the developmental stage (larval size) of the pests observed. Adopting ATs will, in general, reduce the volume of pesticide applied compared to the use of fixed-interval spraying regimes. ATs for botanicals were for some pest species higher than derived from synthetics, and even when they were only moderately effective against pests that were present, they deterred the deposition of further pest eggs onto crop plants. Given that botanical insecticides can affect an array of biological parameters, they have potential to contribute to resistance management strategies (Reddy 2011). Although ATs can be established and adopted within IPM for a given crop, their applicability may have regional limits, due to differences in the pest species present, their crop consumption rates (de Freitas Bueno et al. 2011) and geographical conditions (Reddy 2011). Although this can limit the scope of any study that provides a set of ATs, the approach used is simple and readily transferable to different regions and different crops, and does not rely on prior knowledge to suggest a range of candidate ATs to be trialed. Thus, it can be extended to the benefit of vegetable growers across regions, typically those in developing countries, where botanicals are most widely adopted as alternative pesticides (Amoabeng et al. 2013; Amoabeng et al. 2014). Finally, in the companion study on cauliflower (Shah et al. 2019), the co-occurrence of *S. litura* and *P. xylostella* was not observed and thus the proportional contributions of each were not taken into account in the derivation of ATs, while in the present study ATs were identified when these major pest co-occurred. This approach can thus be used flexibly to tackle individual pest species or complexes of pests.

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Author Contribution Statement

FMS and MR designed the field experiments. FMS and QA conducted the field experiments and gathered the data. SAS provided insecticides, laboratory facilities and assisted in insect identification. FMS and ICWH analyzed the data and wrote the manuscript. MR, AA and MA reviewed the manuscript.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical Approval (Research involving human participants and/or animals)

No specific permits were required for the experiments conducted.

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Figure legends

Figure 1. Overview of seasonal dynamics of insect pests and the timing of experimental trials. Mean numbers of pests present across untreated control plots, for all trials running at each given date, are shown from the start of the first trial until the end of the final trial. (a) Lepidopterans. (b) Aphids. (c) Timing of trials: Dark bars, Bahawalpur trials; Light bars, Multan trials (Supplementary Table 2 gives planting dates at each site).

Figure 2. Effect of insecticides on crop damage and yield. Percentage damage and percentage marketable yields are shown for each treatment and all planting dates at Bahawalpur (a) and Multan (b). Histogram bars are stacked, showing the contributions of each pest species to the crop damage observed. Yields are shown by the jagged lines and the horizontal dotted line illustrates the acceptable marketability criterion of 90%. Control: no spray; NA: NeemAzal weekly spraying (NA-7); NSE: Neem seed extract weekly spraying (NSE-7); VF: Voliam Flexi (chlorantraniliprole + thiamethoxam) sprayed every 5th (VF-5), 10th (VF-10) and 15th day (VF-15).

Figure 3. Relationships between the peak infestation and cumulative insect days (means per plant per replicate).

Figure 4. Relationships between the peak infestation and marketable yield. Yields illustrated in Supplementary Figure 6 are plotted against peak pest densities (Supplementary Figs. 2-5). Data are shown from trials where a given pest was observed.

Table 1. Effects of insecticide treatment and sowing date on the total numbers of pests observed

	Bahawalpur												Multan											
	2015-16						2016-17						2015-16						2016-17					
	Insecticide			Sowing date†			Insecticide			Sowing date			Insecticide			Sowing date			Insecticide			Sowing date		
ANCOVA[1]	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*
Species																								
Spodoptera litura	-	-	-	-	-	-	772.75	5	<0.001*	7852.61	2	<0.001*	480.92	7	<0.001*	1902.94	2	<0.001*	249.09	5	<0.001*	9358.73	5	<0.001*
Spodoptera exigua	205.53	7	<0.001*	151.82	2	<0.001*	435.24	5	<0.001*	1763.12	2	<0.001*	1.57	7	0.980	0.91	2	0.632	106.17	5	<0.001*	187.38	5	<0.001*
Helicoverpa armigera	315.66	7	<0.001*	145.56	2	<0.001*	86.49	5	<0.001*	21.53	2	<0.001*	95.80	7	<0.001*	18.33	2	<0.001*	238.65	5	<0.001*	623.65	5	<0.001*
Plutella xylostella	2186.09	7	<0.001*	369.62	2	<0.001*	578.62	5	<0.001*	305.64	2	<0.001*	1207.12	7	<0.001*	284.67	2	<0.001*	1128.47	5	<0.001*	375.65	5	<0.001*
Trichoplusia orichalcea	238.60	7	<0.001*	249.73	2	<0.001*	-	-	-	-	-	-	47.52	7	<0.001*	28.80	2	<0.001*	-	-	-	-	-	-
Pieris brassicae	-	-	-	-	-	-	-	-	-	-	-	-	2755.84	7	<0.001*	985.79	2	<0.001*	-	-	-	-	-	-
Brevicoryne brassicae	10419.65	7	<0.001*	6221.38	2	<0.001*	1911.15	5	<0.001*	1224.39	2	<0.001*	1701.15	7	<0.001*	21071.26	2	<0.001*	6505.93	5	<0.001*	2328.40	5	<0.001*
Myzus persicae	1503.90	7	<0.001*	1547.26	2	<0.001*	-	-	-	-	-	-	1563.63	7	0.701	8317.19	2	<0.001*	-	-	-	-	-	-
MANOVA[2]																								
Wilks' λ,	0.012			0.055			0.099			0.086			0.040			0.037			0.128			0.032		
$F_{(df)}$	$10.38_{(42,275)}$			$167.11_{(6,58)}$			$5.55_{(25,161)}$			$91.46_{(5,43)}$			$4.496_{(56,306)}$			$179.92_{(8,56)}$			$10.71_{(25,361)}$			$578.34_{(5,97)}$		
P	<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001		

[1] ANOVAs explore effects for each species of pest. ANOVAs assumed Poisson distributed errors and a log-link and the test statistics is the Wald X^2

[2] MANOVAs examine effects on the composition of pests present.

† Supplementary Table 2 gives the exact dates of sowing for each site in each year.

* Because several ANCOVA tests were carried within years and sites, significance was re-evaluated within each year using the false discovery rate (FDR) procedure. Values that remained significant following this correction are indicated with an asterisk, other results are considered non-significant.

- Not observed.

Table 2. Effects of insecticide treatment on weekly numbers of each pest species observed

Species	**Sowing date**	**District**	**Insecticide**			**Sample time**			**Insecticide × Sample time interaction**		
			F-ratio[1]	*df*	*P*	*F-ratio*	*df*	*P*	*F-ratio*	*df*	*P*
Brevicorynae brassicae	18/10/2015	Bahawalpur	31.97	7,16	<0.001*	309.71	7,112	<0.001*	9.39	49,112	<0.001*
	10/11/2015	Bahawalpur	502.73	7,16	<0.001*	216.37	9,144	<0.001*	57.52	63,144	<0.001*
	04/12/2015	Bahawalpur	19.70	7,16	<0.001*	71.34	3,48	<0.001*	5.70	21,48	<0.001*
	02/12/2016	Bahawalpur	15.00	5	0.010*	-	-	-	-	-	-
	20/01/2016	Multan	43.76	7,16	<0.001*	463.70	4,64	<0.001*	7.38	28,64	<0.001*
	20/02/2016	Multan	12.68	7,16	<0.001*	843.10	4,64	<0.001*	7.72	28,64	<0.001*
	02/12/2016	Multan	408.60	5,12	<0.001*	304.26	7,84	<0.001*	34.43	35,84	<0.001*
	23/12/2016	Multan	1537.45	5,12	<0.001*	235.71	7,84	<0.001*	38.90	35,84	<0.001*
Spodoptera litura[2]	15/03/2016†	Multan	11.76	7	0.038*	-	-	-	-	-	-
	10/09/2016†	Bahawalpur	8.90	5	0.113*	-	-	-	-	-	-
	02/12/2016	Bahawalpur	6.64	5,12	0.003*	244.32	5,60	<0.001*	3.80	25,60	0.005*
	25/12/2016	Bahawalpur	13.59	5,12	<0.001*	62.67	3,36	<0.001*	4.85	15,36	0.006*
	20/02/2017†	Multan	9.90	5	0.078*	-	-	-	-	-	-
	10/03/2017†	Multan	11.57	5	0.041*	-	-	-	-	-	-
Myzus persicae	10/11/2015	Bahawalpur	15.43	7,16	<0.001*	43.18	3,48	<0.001*	7.91	21,48	<0.001*
	04/12/2015	Bahawalpur	79.05	7,16	<0.001*	166.00	3,48	<0.001*	15.36	21,48	<0.001*
	20/01/2016†	Multan	8.11	7	0.300	-	-	-	-	-	-
	20/02/2016	Multan	3.88	7,16	0.012*	15.33	2,32	0.001*	3.95	14,32	0.009*
Plutella xylostella	18/10/2015†	Bahawalpur	20.77	7	0.004*	-	-	-	-	-	-
	10/11/2015†	Bahawalpur	20.72	7	0.004*	-	-	-	-	-	-
	04/12/2015	Bahawalpur	56.25	7,16	<0.001*	90.78	4,64	<0.001*	6.73	28,64	<0.001*
	20/01/2016†	Multan	20.55	7	0.004*	-	-	-	-	-	-
	20/02/2016†	Multan	16.92	7	0.018*	-	-	-	-	-	-
	15/03/2016†	Multan	16.22	7	0.023*	-	-	-	-	-	-
	02/12/2016	Bahawalpur	170.38	5,12	<0.001*	71.90	6,72	<0.001*	11.12	30,72	<0.001*
	02/12/2016	Multan	203.82	5,12	<0.001*	61.82	7,84	<0.001*	11.44	35,84	<0.001*
	23/12/2016	Multan	35.43	5,12	<0.001*	55.95	7,84	<0.001*	6.55	35,84	<0.001*
	25/12/2016	Bahawalpur	60.39	5,12	<0.001*	52.33	6,72	<0.001*	4.42	30,72	<0.001*
	23/01/2017	Multan	23.06	5,12	<0.001*	8.91	4,48	<0.001*	6.78	20,48	<0.001*
	01/02/2017	Multan	43.01	5,12	<0.001*	23.54	7,84	<0.001*	4.31	35,84	<0.001*
	20/02/2017†	Multan	14.34	5	0.014*	-	-	-	-	-	-
	10/03/2017†	Multan	14.40	5	0.013*	-	-	-	-	-	-
Spodoptera exigua	18/10/2015	Bahawalpur	13.69	7,16	<0.001*	47.99	2,32	<0.001*	10.95	14,32	<0.001*

	10/11/2015†	Bahawalpur	18.95	7	0.008*	-	-	-	-	-	-
	04/12/2015	Bahawalpur	13.23	7,16	<0.001*	21.80	3,48	<0.001*	8.69	21,48	<0.001*
	20/02/2016	Multan	9.081	7,16	<0.001*	7.72	2,32	0.004*	5.91	14,32	<0.001*
	02/12/2016	Bahawalpur	1.59	5,12	0.236	234.20	1,12	<0.001*	1.80	5,12	0.186
	25/12/2016	Bahawalpur	2.00	5,12	0.151	69.34	1,12	<0.001*	6.44	5,12	0.004*
	20/02/2017†	Multan	9.90	5	0.078*	-	-	-	-	-	-
Pieris brassicae	15/03/2016†	Multan	13.11	7	0.069	-	-	-	-	-	-
Helicoverpa armigera	18/10/2015†	Bahawalpur	20.88	7	0.004*	-	-	-	-	-	-
	10/11/2015†	Bahawalpur	19.99	7	0.006*	-	-	-	-	-	-
	04/12/2015†	Bahawalpur	20.30	7	0.005*	-	-	-	-	-	
	20/01/2016	Multan	24.31	7,16	<0.001*	4.93	3,48	0.011*	6.75	21,48	<0.001*
	20/02/2016†	Multan	19.03	7	0.008*	-	-	-	-	-	-
	10/09/2016	Bahawalpur	32.35	5,12	<0.001*	13.36	3,36	<0.001*	4.95	15,36	<0.001*
	02/12/2016	Bahawalpur	6.81	5,12	0.003*	7.18	2,24	0.007*	6.79	10,24	<0.001*
	02/12/2016	Multan	31.74	5,12	<0.001*	4.62	5,60	0.008*	1.70	25,60	0.097
	23/12/2016	Multan	14.56	5,12	<0.001*	13.68	4,48	<0.001*	4.02	20,48	<0.001*
	25/12/2016	Bahawalpur	23.86	5,12	<0.001*	58.94	2,24	<0.001*	9.14	10,24	<0.001*
	23/01/2017	Multan	12.52	5,12	<0.001*	89.26	6,72	<0.001*	4.87	30,72	<0.001*
	01/02/2017	Multan	34.73	5,12	<0.001*	40.79	7,84	<0.001*	3.23	35,84	0.001*
	20/02/2017	Multan	20.72	5,12	<0.001*	14.48	3,36	<0.001*	4.87	15,36	0.002*
	10/03/2017	Multan	32.61	5,12	<0.001*	0.53	2,24	0.590	1.39	10,24	0.250
Trichoplusia orichalcea	18/10/2015†	Bahawalpur	18.66	7	0.009*	-	-	-	-	-	-
	10/11/2015†	Bahawalpur	17.57	7	0.014*	-	-	-	-	-	-
	04/12/2015	Bahawalpur	42.83	7,16	<0.001*	22.71	4,64	<0.001*	11.45	28,64	<0.001*
	20/02/2016†	Multan	20.03	7	0.005*	-	-	-	-	-	-
	15/03/2016†	Multan	17.09	7	0.017*	-	-	-	-	-	-

Results are shown for pest species in order of decreasing abundance.

[1] The test statistic is the *F*-ratio when both numerator and denominator degrees of freedom are given, otherwise values are of the Friedman's test statistic.

[2] *Spodoptera litura* data were for all larval size classes combined: see Supplementary Table 3 for separate analyses.

†Insect count data were normalized by transformation and, in some instances, excluding dates with zeros (see Methods); when normalization could not be achived, data were reanalysed using non-parametric Friedman's tests. As Friedman's tests were performed on seasonal totals (total numbers per plant across sampling dates), effects of within-seasons sampling times and their interaction with insecticide treatment were not assessed. In other cases, repeated measures ANOVAs were used to explore effects of insecticides on pest abundance across multiple sampling dates.

*Where several statistical tests were carried out for a species, significance was re-evaluated using the FDR procedure. *P*-values remaining significant following this correction are indicated with an asterisk and other results are considered non-significant. A multiple comparisons test was also carried out across all 59 results: this led to the same conclusions as the species-by-species corrections.

Fig. 1

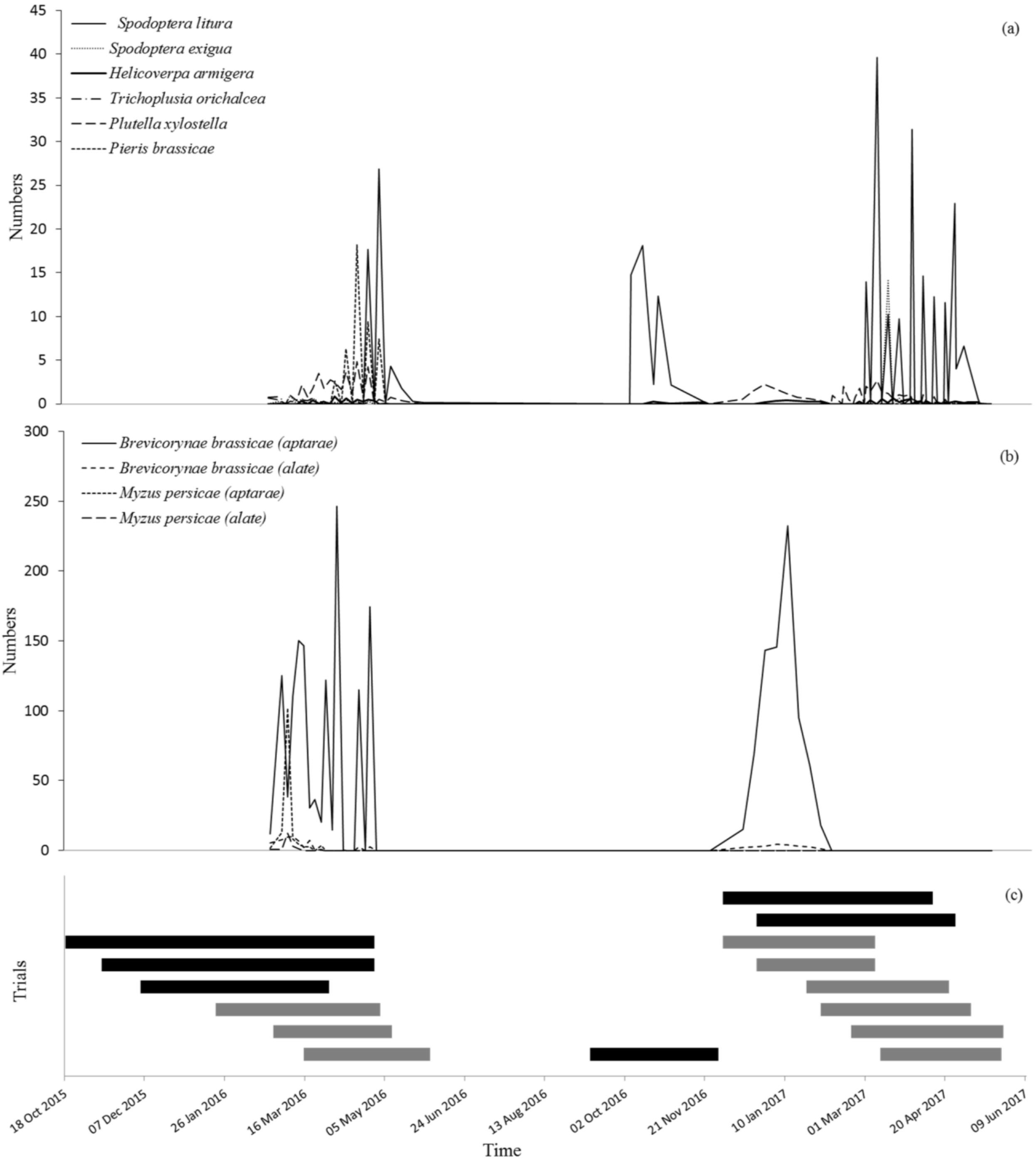

(a)
Spodoptera litura
Spodoptera exigua
Helicoverpa armigera
Trichoplusia orichalcea
Plutella xylostella
Pieris brassicae
Numbers
45
40
35
30
25
20
15
10
5
0
(b)
Brevicorynae brassicae (aptarae)
Brevicorynae brassicae (alate)
Myzus persicae (aptarae)
Myzus persicae (alate)
Numbers
300
250
200
150
100
50
0
(c)
Trials
18 Oct 2015
07 Dec 2015
26 Jan 2016
16 Mar 2016
05 May 2016
24 Jun 2016
13 Aug 2016
02 Oct 2016
21 Nov 2016
10 Jan 2017
01 Mar 2017
20 Apr 2017
09 Jun 2017
Time

Fig. 2

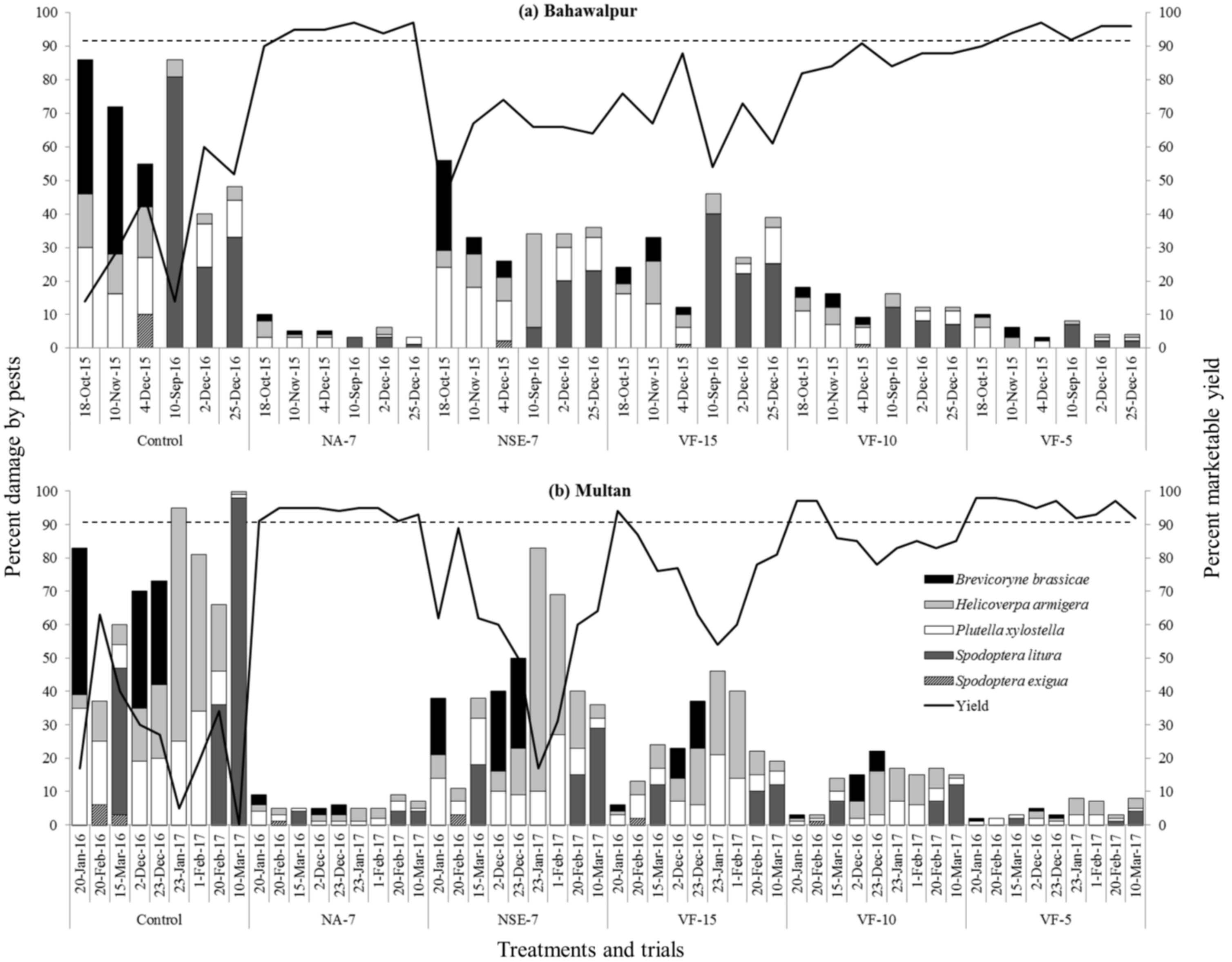

(a) Bahawalpur
(b) Multan
Percent damage by pests
Percent marketable yield
0
10
20
30
40
50
60
70
80
90
100
18-Oct-15
10-Nov-15
4-Dec-15
10-Sep-16
2-Dec-16
25-Dec-16
20-Jan-16
20-Feb-16
15-Mar-16
2-Dec-16
23-Dec-16
23-Jan-17
1-Feb-17
20-Feb-17
10-Mar-17
Control
NA-7
NSE-7
VF-15
VF-10
VF-5
Brevicoryne brassicae
Helicoverpa armigera
Plutella xylostella
Spodoptera litura
Spodoptera exigua
Yield
Treatments and trials

Fig. 3

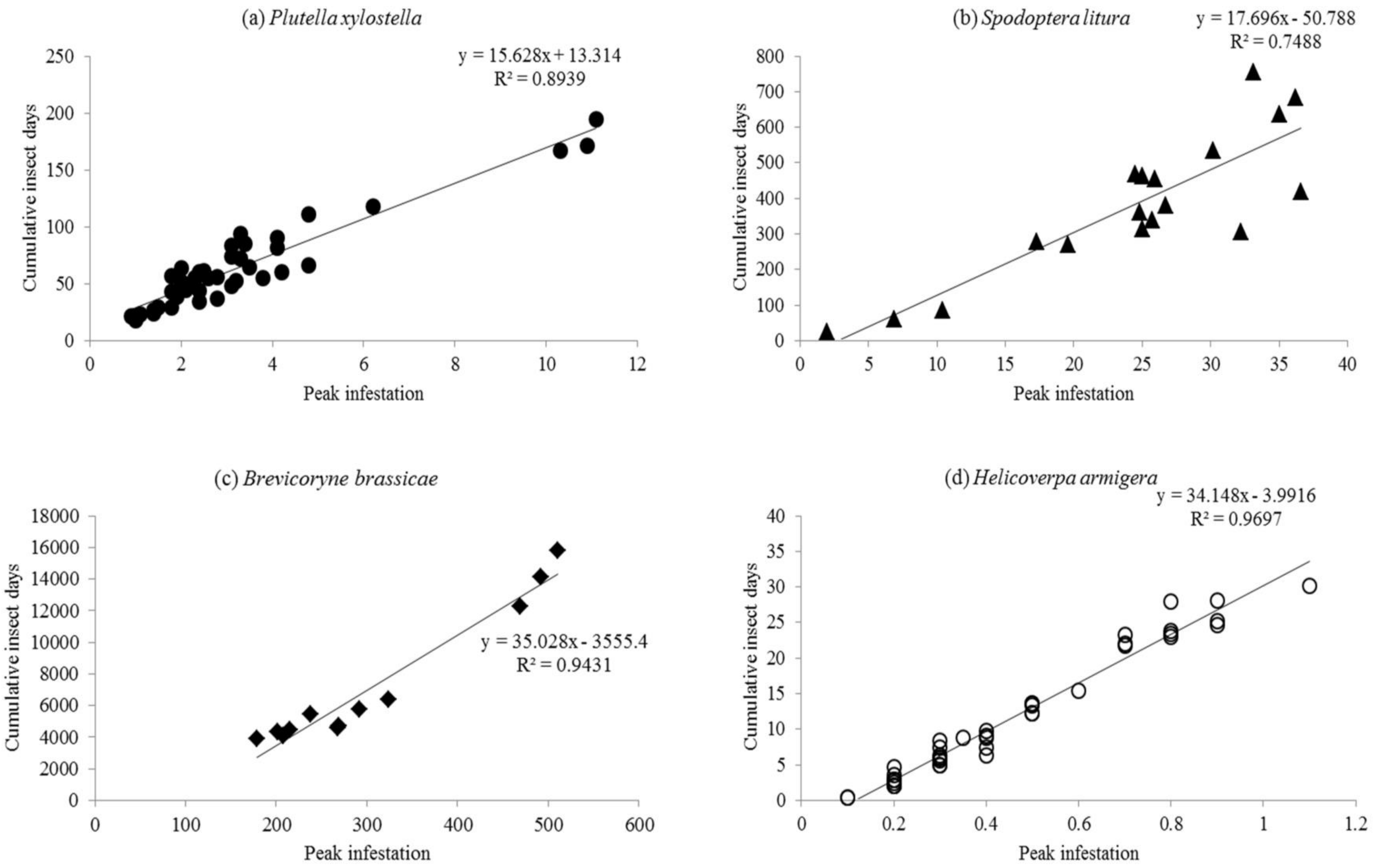
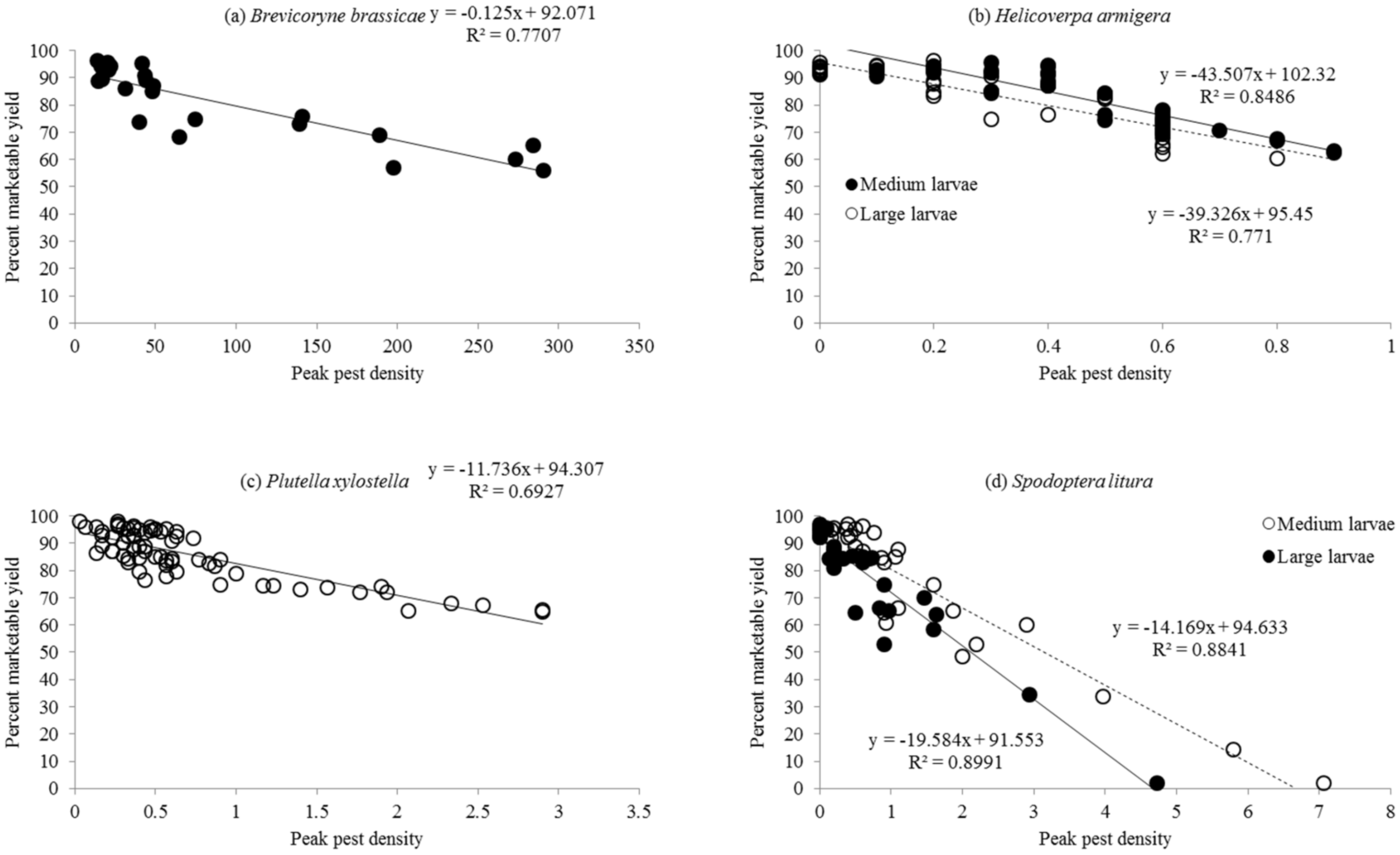

(a) *Plutella xylostella*
y = 15.628x + 13.314
R² = 0.8939
Cumulative insect days
Peak infestation
0
50
100
150
200
250
0
2
4
6
8
10
12
(b) *Spodoptera litura*
y = 17.696x - 50.788
R² = 0.7488
Cumulative insect days
Peak infestation
0
100
200
300
400
500
600
700
800
0
5
10
15
20
25
30
35
40
(c) *Brevicoryne brassicae*
y = 35.028x - 3555.4
R² = 0.9431
Cumulative insect days
Peak infestation
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
0
100
200
300
400
500
600
(d) *Helicoverpa armigera*
y = 34.148x - 3.9916
R² = 0.9697
Cumulative insect days
Peak infestation
0
5
10
15
20
25
30
35
40
0
0.2
0.4
0.6
0.8
1
1.2

Fig. 4


(a) Brevicoryne brassicae
y = -0.125x + 92.071
R² = 0.7707
Percent marketable yield
Peak pest density
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
250
300
350
(b) Helicoverpa armigera
y = -43.507x + 102.32
R² = 0.8486
● Medium larvae
○ Large larvae
y = -39.326x + 95.45
R² = 0.771
Percent marketable yield
Peak pest density
0
0.2
0.4
0.6
0.8
1
(c) Plutella xylostella
y = -11.736x + 94.307
R² = 0.6927
Percent marketable yield
Peak pest density
0
0.5
1
1.5
2
2.5
3
3.5
(d) Spodoptera litura
○ Medium larvae
● Large larvae
y = -14.169x + 94.633
R² = 0.8841
y = -19.584x + 91.553
R² = 0.8991
Percent marketable yield
Peak pest density
0
1
2
3
4
5
6
7
8