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Original article (Orijinal araştırma)

Effect of botanicals and synthetic insecticides on *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae)

Bitkisel ve sentetik insektisitlerin *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae) üzerine etkileri

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Summary

Cabbage white butterfly, *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae), is one of the severe insect pests of cabbage crop which causes remarkable quantitative or qualitative crop losses. The effect of different new chemistry insecticides (thiamethoxam 25% SP, acetamiprid 20% SP and pyriproxyfen 10.8% EC) and four botanical extracts, *Aloe vera* (L.) Burm. f. leaves, grapefruit (*Citrus* × *paradisi* Macfad.) bark, spearmint (*Mentha spicata* L.) leaves and neem (*Azadirachta indica* A. Juss.) leaves, on the feeding behavior, larval growth and mortality of *P. brassicae* was studied at University of Sargodha (Pakistan). The study showed that neem extracts at 7% had a significant effect on growth parameters of *P. brassicae*. The relative growth rate was the lowest (3.05±0.27 mg/mg/d) when neem extract was applied at 7% and higher (8.59±1.38 mg/mg/d) when grapefruit extract was applied at 5% after the control treatment. In comparison to control treatment, relative feed consumption rate of *P. brassicae* and the efficiency of conversion of ingested food decreased to 66 and 58%, respectively, after the application of neem extract at 7%. Neem extracts at 7% caused up to 65% larval mortality. The neem extract (7%) in combination with pyriproxyfen also caused the significant stress on the larvae. So, neem extracts at 7% alone or in combination with insecticides can be used for control of *P. brassicae* in vegetable crops for a safer food supply.

Keywords: Botanical extracts, cabbage white caterpillar, feeding indices, insecticides, *Pieris brassicae*

Özet

Lahana beyaz kelebeği, *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae), lahanada dikkate değer nicel veya nitel ürün kayıplarına neden olan en önemli zararlılardan biridir. Bu çalışmada Sargodha Üniversitesi (Pakistan)'nde, farklı yeni kimyasal insektisitlerin (thiamethoxam 25% SP, acetamiprid 20% SP ve pyriproxyfen 10.8% EC) ve *Aloe vera* (L.) Burm. f. yaprakları, greyfurt (*Citrus* × *paradisi* Macfad.) kabuğu, nane (*Mentha spicata* L.) yaprakları ve neem (*Azadirachta indica* A. Juss.) yapraklarından elde edilen dört bitki ekstraktının, *P. brassicae*'nin beslenme davranışlarına, larva gelişimine ve öldürücü etkileri araştırılmıştır. Çalışmada *P. brassicae*'nin gelişme parametrelerinde en önemli etkiyi %7'lik neem ekstaktı göstermiştir. Göreceli büyüme oranı %7'lik neem ekstraktı uygulandığında en düşük (3.05±0.27 mg/mg/d) olurken, kontrol uygulamasından sonra %5 oranında greyfurt ekstraktı uygulandığında daha yüksek (8.59±1.38 mg/mg/d) olmuştur. Kontrol uygulamasına kıyasla, *P. brassicae*'nin göreceli yem tüketimi oranı ve yutulan yiyeceklerin dönüşüm verimliliği %7'lik neem ekstraktı uygulandıktan sonra sırasıyla %66 ve %58'e düşmüştür. Neem ekstraktının %7'lik uygulaması, %65'e kadar larva ölümüne sebep olmuştur. Ayrıca, neem ekstraktı (%7) ile pyriproxyfen kombinasyonu, larvalar üzerinde önemli strese neden olmuştur. Bu nedenle, tek başına veya insektisitlerle kombinasyon halinde %7 oranında neem ekstraktı, sebze üretiminde *P. brassicae*'nin kontrolünde daha güvenli bir gıda için kullanılabileceği söylenebilir.

Anahtar sözcükler: Bitkisel ekstraktlar, lahana beyaz kelebeği, beslenme göstergesi, insektisitler, *Pieris brassicae*

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Introduction

The reduction in cabbage production and yield is strongly related with insect herbivory (Tolman et al., 2004). Among many insect herbivores, cabbage white butterfly, *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae), is a serious insect pest which can heavily infest cabbage crops (Hasan & Ansari, 2010). This cosmopolitan pest causes severe damage to 15 plant species including cabbage, cauliflower, mustard, radish, rape and turnip (Hwang et al., 2008). This pest damages all plant stages in cabbage crops, i.e., seedlings, vegetative and flowering stages (Ullah et al., 2016b). Young caterpillars of *P. brassicae* are gregarious leaf feeders (Hasan & Ansari, 2011). The larvae of *P. brassicae* feed on all plant parts including leaves, twigs, fruits and seeds of cabbage and cauliflower (Siraj, 1999). A single larva can consume up to 74 to 80 cm^2 of leaf and causes serious damage to the host plant (Younas, et al., 2004). *Pieris brassicae* causes more than 40% yield loss in different vegetable crops in India annually (Ali & Rizvi, 2007). Extreme infestations of *P. brassicae* completely destroy the plant foliage and ultimately kill the plant (Hasan & Ansari, 2010).

Usually, farmers are dependent on synthetic insecticides to suppress *P. brassicae* infestations in cabbage crops (Ullah et al., 2016b). The frequent use of synthetic chemicals has resulted in resistance to these insecticides. Consequently, it is now difficult to control this pest using existing synthetic insecticides (Thomas, 1999). The injudicious use of insecticides has led to many biological and environmental problems, such as toxicity to non-targeted plants, insects and other organisms, environmental degradation, and human health hazards (Badshah et al., 2015; Zahid et al., 2016). Given the destructive nature of synthetic chemical insecticides, it is critical to develop safe and environment friendly resources for better and safer pest management (Rangad et al., 2014).

Plant based insecticides (botanicals) are well known for their insecticidal and insect repellent characters and lower toxicity to the environment (Zahid et al., 2016). The use of botanicals for control of *P. brassicae* can be effective, and safer than synthetic chemicals due to their lower residual effect on non-target organisms (Endersby & Morgan, 1991; Raguraman & Kannan, 2014). Crude plant extracts may alter the behavioral and physiological aspects of insects, which may result in a reduction of insect pest infestations in crops (Sharma & Gupta, 2009). Botanical insecticides are highly preferred because they are less expensive and easily available from commonly grown plants (Salim & Abed, 2015). Furthermore, botanicals usually have a slight impact on the biological activity of natural enemies and they can be safely combined with other control practices (Zahid et al., 2016). Herbivore nutritional indices are negatively affected by the application of botanicals or plant oils with a reduction in feeding and growth indices after the treatment (Huang et al., 2000; Pavela et al., 2009; Zapata et al., 2009; Taghizadeh et al., 2014). *Reynoutria* plant extracts can reduce the nutritional indices of the last instars of *Spodoptera littoralis* (Boisduval, 1833) larvae (Pavela et al., 2008). Plant extracts can lower the relative growth rate (RGR), relative consumption rate (RCR) and efficiency of conversion of ingested food (ECI), and lead to retarded larval growth and smaller pupae. These effects result into lowered fecundity and longevity of the adult insects with susceptibility to certain diseases and natural enemies (Khosravi et al., 2010).

Botanicals are comparatively safer to the parasitoids like *Cotesia glomerata* (L., 1758), which voraciously parasitizes the *P. brassicae* caterpillar (Ullah et al., 2016c), when compared to synthetic insecticides (Yi et al., 2016). Botanicals are even less toxic to the developing larvae of parasitoids in the host insects exposed to them (Tang et al., 2002). In addition, the majority of botanicals contain a diverse mix of active compounds and thus do not result in pest resistance (Pavela, 2011).

Given the above problems of chemical use in food crops, the current study aimed to evaluate the efficacy of different new chemistry insecticides and some plant extracts alone and in combination on *P. brassicae* mortality and growth performance.

Material and Methods

The experiment was conducted in Entomology Laboratory, University College of Agriculture, University of Sargodha, Pakistan in 2016. Caterpillars of *P. brassicae* were collected from the cabbage fields near the university and brought to the laboratory for rearing under controlled conditions at 25±2ºC and 65±2% RH. For the maintenance of the insect culture, fresh cabbage leaves (*Brassica oleracea* L.) were provided daily. Second instar caterpillars, from the next generation, were used for the further experiment.

Insecticides and plant extracts

Three commercially available synthetic insecticides, thiamethoxam 25% SP, acetamiprid 20% SP, pyriproxyfen 10.8% EC (Arysta Life Sciences, Karachi, Pakistan), at field recommended doses (0.024, 0.06 and 0.05 g/100 ml, respectively) and four plant extracts, *Aloe vera* (L.) Burm. f. leaves, grapefruit (*Citrus* × *paradisi* Macfad.) bark, spearmint (*Mentha spicata* L.) leaves and neem (*Azadirachta indica* A. Juss.) leaves at 5 and 7%, were used.

Preparation of plant extracts

The insecticide free plant material of the selected plant species was collected from the field and washed with distilled water. The required plant parts were sun dried. The dried plant materials were ground to powder with an electrical grinder. Five g of each plant powdered samples were placed in conical flasks (250 ml) along with 100 ml of distilled water. The flasks were placed on the heating magnetic stirrer (AM4, Velp Scientifica, Usmate, Italy) for 4 h. The solid residues were removed using muslin cloth. The liquid extracts were then filtered (Whatman No. 1) using a vacuum suction assembly (Sparmax, Taipei, Taiwan). The extracts were dried in a rotary evaporator (HB Digital, Heidolph, Schwabach, Germany) at 60ºC under vacuum. These dried plant extracts were brought to constant weight in hot air oven (60ºC). The extracts were stored at 5ºC until used. Concentrations of 5 and 7% were prepared in water for experimental evaluation.

Assay of feeding indices and mortality

Fresh cabbage leaves were cut according to fit Petri dishes. A leaf-dip bioassay was used to check the efficacy of each chemical against *P. brassicae* caterpillars. The cut cabbage leaves were dipped in 100 ml prepared solutions of the diluted botanicals and synthetic insecticides for 10 s and air dried for 30 min on filter papers before offering this leave to second instar caterpillars under ventilation. Leaves and *P. brassicae* caterpillars were weighed before transferring them into the experimental arena. The experiment was replicated four times with five larvae in each replicate. Water was used as a control treatment.

Data was recorded at 12-h intervals after the application of insecticides and botanicals. Caterpillar, fresh diet and feces weights for each replicate was recorded to estimate the feeding indices for 4 d. A high precision balance (±0.1 mg) was used to weigh all materials. The mortality of *P. brassicae* caterpillars was also recorded to check the toxicity of the treatments. The corrected percent mortality of *P. brassicae* caterpillars was obtained using Abbott's formula (Abbott, 1925). In a subsequent experiment, the most effective synthetic insecticide (pyriproxyfen) and botanical (neem 7%) were combined at ratios of 1:2 and 2:1 (insecticide: botanical) to evaluate the efficacy of these combinations. Growth indices parameters were calculated by the formulas of Ullah et al. (2016a).

Relative growth rate (RGR) = B-A/B x d

Relative consumption rate (RCR) = D/B x d

Efficiency of conversion of ingested food (ECI) = B/D x 100

Where, A is the mean weight (g) of the insects on the fourth day, B is the original mean weight of insects (g) and D is the food biomass ingested (g) per insect.

Data analysis

The mortality data was log-transformed to achieve normality before analysis. RGR, RCR, ECI and mortality data were subjected to analysis of variance and Tukey's HSD test was used to compare the means with Minitab 16.1 software to check the direct and indirect effects of the botanicals and synthetic chemicals on the larval performance. The original mean values are given below.

Results

The treatments had significant effects (P < 0.001) on RGR (F = 84.9, df = 13 and P < 0.001), RCR (F = 53.7, df = 13 and P < 0.001) and ECI (F = 64.5, df = 13 and P < 0.001) of *P. brassicae* (Table 1). Time interval was only significant for RCR and ECI. However, the interaction between treatment and time interval was highly significant for all parameters (F = 3.81, 2.32 and 2.68 for RGR, RCR and ECI, respectively, at P < 0.001 and df = 39).

Table 1. Growth indices of *Pieris brassicae* at different time intervals after the application of different synthetic insecticides and botanicals

			RGR		RCR		ECI	
Source	DF	MS	F value	P value	F value	P value	F value	P value
Treatments (A)	13	119.71	84.92	<0.001	53.67	<0.001	64.48	<0.001
Time (B)	3	0.48	0.34	>0.05	4.75	<0.05	21.84	<0.001
A x B	39	5.37	3.81	<0.001	2.32	<0.001	2.68	<0.001
Residual	168	1.41						
Total	223							

P < 0.05, significant; P < 0.001, highly significant; P > 0.05, non-significant; RGR, relative growth rate (mg/mg body weight/d); RCR, relative consumption rate (mg/mg body weight/d); ECI, efficiency of conversion of ingested food (%); DF, degree of freedom; MS, mean square.

The percent mortality data showed that the neem extract at 7% was lethal to *P. brassicae* caterpillars with the maximum mortality of 65% (Figure 1). Similarly, the combined action of pyriproxyfen with 7% neem extract also caused significant mortality of *P. brassicae* caterpillars. The insecticide and botanical combinations (1:2 and 2:1) caused 60.9% and 58.8% mortality of *P. brassicae* caterpillars, respectively. The least effective botanical was grapefruit bark extract at 5%, which caused only 26.6% mortality of *P. brassicae* caterpillars.

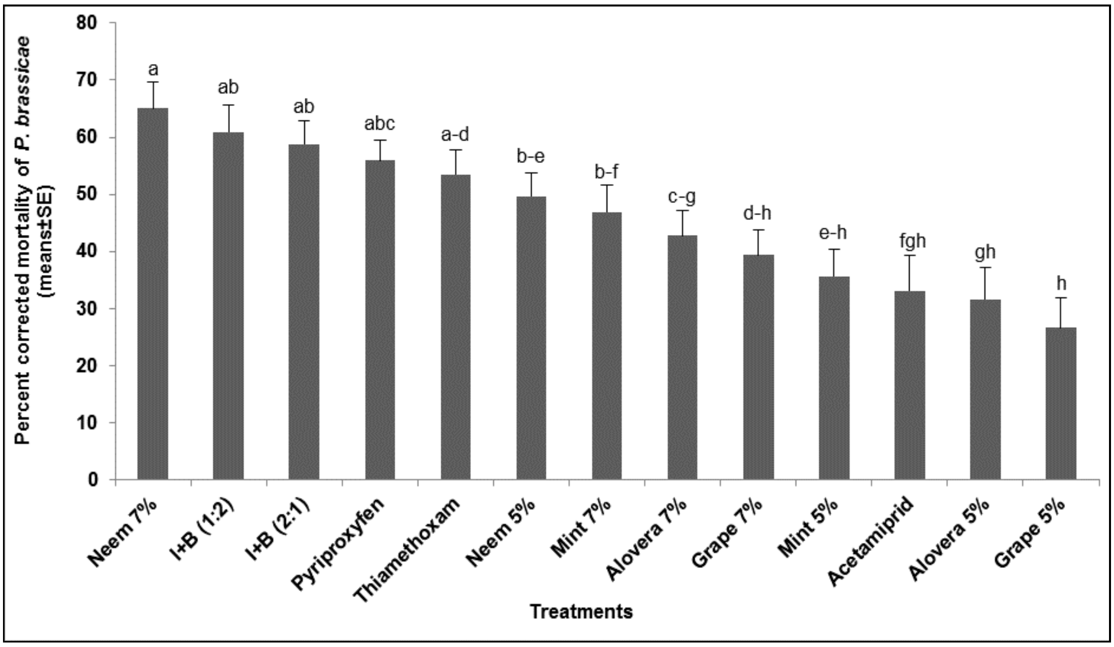

Figure 1. Percent mortality of *Pieris brassicae* after the application of different botanicals and synthetic chemicals (alone and in combination) [I+B, insecticide (pyriproxyfen) + botanical (neem 7%)], means sharing similar letters are not significantly different from each other.

The results indicated that neem leaf extract (7%) also had significant negative effects on growth parameters of *P. brassicae* (Figures 2 to 4). The RGR was the lowest (3.05±0.27 mg/mg body weight/d) with 7% neem extract application and the highest (8.59±1.37 mg/mg body weight/d) with grapefruit bark extract at 5% (Figure 2). Similar effects of 7% neem extracts were seen for ECR and ECI (Figures 3 & 4). In comparison to control treatment, the relative consumption rate of *P. brassicae* was lowered by 66% with the application of 7% neem extract (Figure 3). Similarly, the ECR decreased by 57.6% in the same treatment (Figure 4). Overall, RGR, RCR, and ECI were higher in control treatment. Both combinations of pyriproxyfen and 7% neem extract showed significant negative effects on the growth of *P. brassicae*.

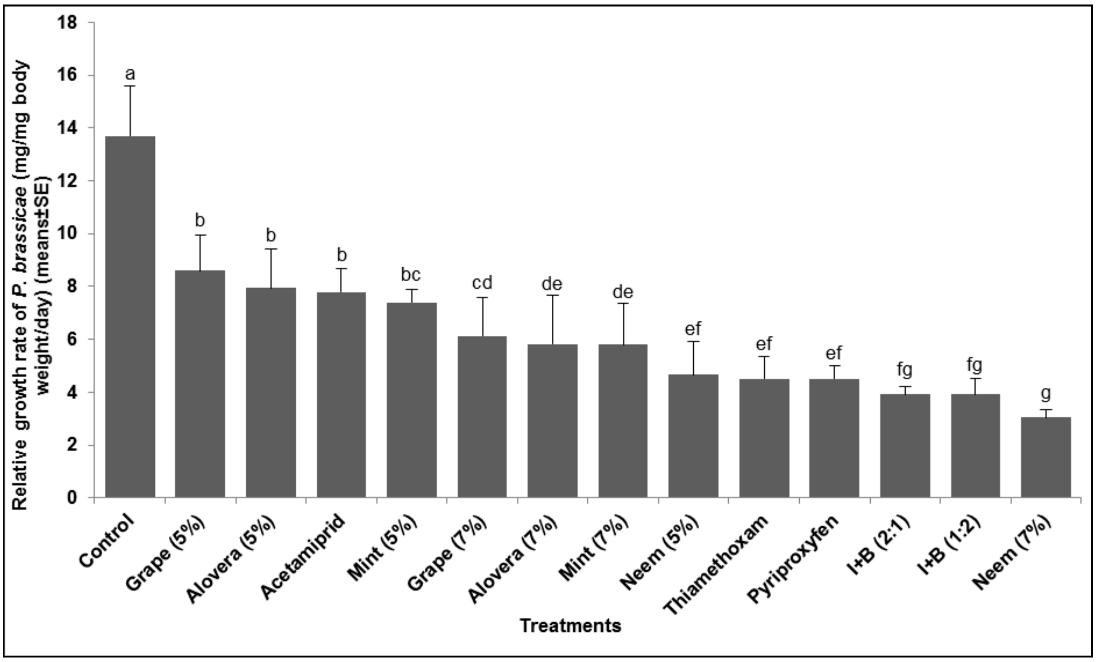

Figure 2. Relative growth rate (RGR) of *Pieris brassicae* after the application of different botanicals and synthetic chemicals (alone and in combination) [I+B, insecticide (pyriproxyfen) + botanical (neem 7%)], means sharing similar letters are not significantly different from each other.

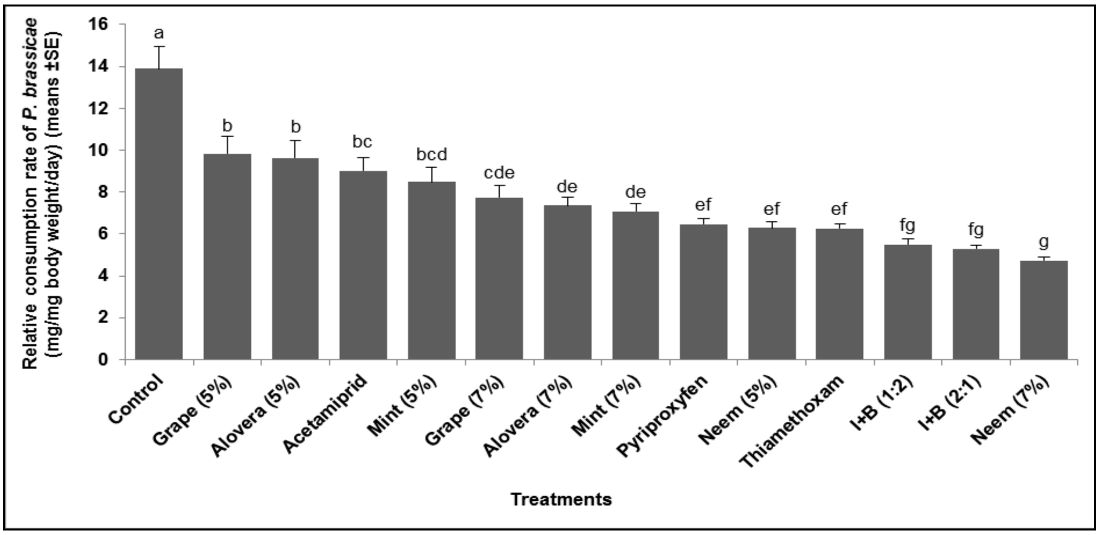

Figure 3. Relative consumption rate of *Pieris brassicae* after the application of different botanicals and synthetic chemicals (alone and in combination) [I+B, insecticide (pyriproxyfen) + botanical (neem 7%)], means sharing similar letters are not significantly different from each other.

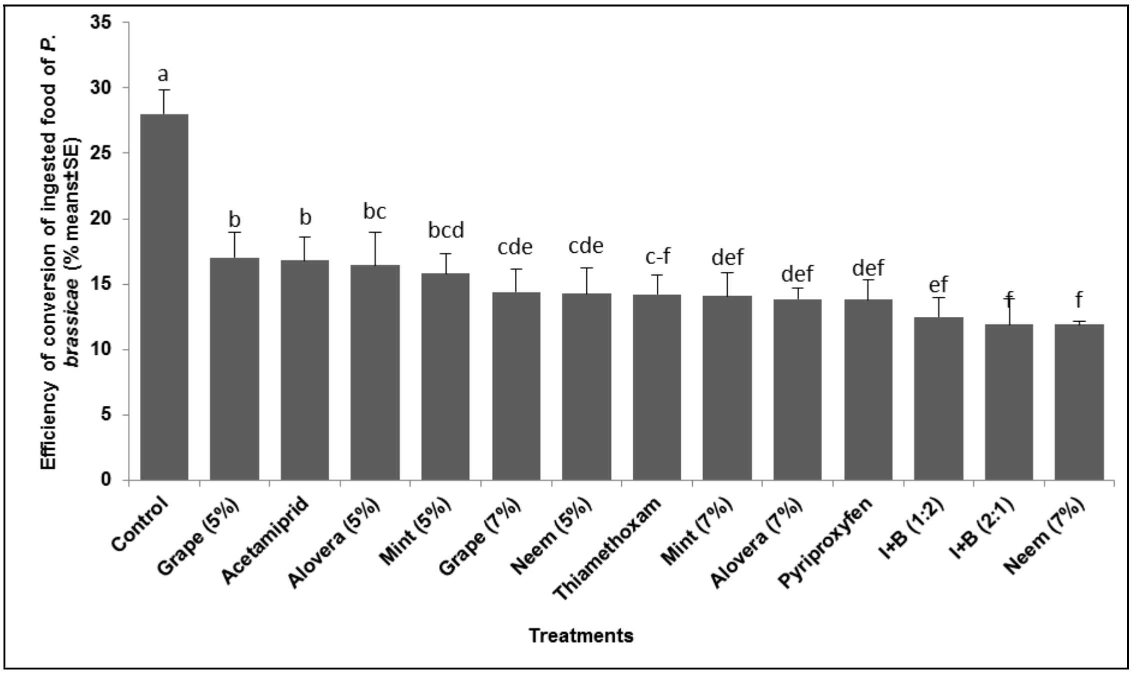

Figure 4. Efficiency of conversion of ingested food of *Pieris brassicae* after the application of different botanicals and synthetic chemicals (alone and in combination) [I+B, insecticide (pyriproxyfen) + botanical (neem 7%)], means sharing similar letters are not significantly different from each other.

Discussion

Natural plant products or botanicals can be an excellent substitute for synthetic pesticides by reducing the health and environment hazards associated with synthetics (Mohan et al., 2011). Application of natural insecticides could be a promising approach to decrease the overall use of synthetic insecticides (Pavela, 2007; Dayan et al., 2009).

In neem extracts, methyl petroselinate, hexadecamethyl cyclooctasiloxane, methyl isoheptadecanoate, oxalic acid, 2-ethylhexyl tetradecyl ester, butyl palmitate, heptacosane 7-hexyl, and eicosane are the major biologically active chemical compounds (Hossain et al., 2013). *Mentha* spp. mainly contain 1,8-cineole, limonene, carvone, linalyl acetate, linalool, menthone, menthol, piperitenone oxide and methyl acetate (Gracindo et al., 2006). The important biologically active compounds in *A. vera* are aloe-emodin, anthranol, aloetic-acid, barbaloin, emodin, isobarbaloin and esters of cinnamic acid (Moghaddasi & Verma, 2011). The grapefruit bark contains many active biocompounds including flavonoids, saponin, alkaloids, cardenolide aglycone, cardiac glycoside, tannin and terpenoids (Olabinri et al., 2014). Among these plant metabolites, many natural plant products are known for their insecticide, fumigant, repellent and antifeedant actions (Shaaya et al., 1997).

Botanicals possess insecticidal properties due to physical action and muscular poisoning. Forty-seven plant species have been listed for their toxicity to different insect species (Talukder, 2006). In our study, neem leaf extracts at 7% caused the highest larval mortality of the tested botanicals and synthetic chemicals. Neem extracts contain about 100 bioactive compounds. Among these, triterpenes (limonoids) are the most important, causing 90% of the effects on most of the insect pests. It has already been reported that neem extracts have useful insecticidal properties against many lepidopteran insect pests. Larval and nymphal mortalities in many other insect groups have also been reported (Warthen, 1989; Campos et al., 2016). Although, food consumption was lower on leaves treated with plant extracts, considerable larval mortality was recorded in all treatments, with the toxic effect of plant extracts greatest for neem extract at 7%. This shows that higher concentrations of plant extracts can cause high larval mortality even with only a small amount of treated food being consumed (Leatemia & Isman, 2004). *Capparis spinosa* L. leaf extracts caused 100% mortality of *S. littoralis* larvae through antifeeding as well as insecticidal effects (Ladhari et al., 2013). Similarly, *Allium indica* L. (garlic) and *Melia azedarach* L. extract effectively reduced *P. brassicae* infestations in cabbage crops (Przybyszewski, 1993; Khan & Siddiqui, 1994; Grisakova et al., 2006 and Sharma & Gupta, 2009). In the current study, neem leaf extract at 7% gave significant mortality of *P. brassicae* as well as feeding disturbances to the caterpillars and reducing the growth and development of insect larvae.

Any substance reducing the food ingestion by insects is categorized as an antifeedant and generally has adverse effects on insect feeding behavior (Hummelbrunner & Isman, 2001), which was exhibited by our extracts, especially neem leaf extract at 7%, which had a significant antifeeding effect. Antifeedants can be described as allomone inhibiting insect feeding and do not kill the exposed insect pests directly but rather, they affect their developmental potential considerably by acting as a phagorepellent (Lakshmanan et al., 2012). Many botanicals offer antifeedant as well as toxic effects against *Spodoptera litura* (Fabricius, 1775) (Ulrichs et al., 2008; Arivoli & Samuel, 2012). Similarly, extracts of *Trichilia prieureana* A. Juss*., Trichilia roka* (Forssk.) Chiov. and *Trichilia connaroides* (Wight & Am.) Bentv. seeds gave high levels of feeding inhibition against *Spodoptera frugiperda* (J. E. Smith, 1797) (Mikolajczak & Reed, 1987). Leaf extracts of *Justicia vasica* L. and *C. spinosa* also caused strong feeding inhibition in *S. littoralis* (Sadek, 2003; Ladhari et al., 2013). Charleston et al. (2005) also reported aqueous extracts of *M. azedarach* and *A. indica* as antifeedants and growth inhibitors for the larvae of *Plutella xylostella* (L., 1758). From the above studies, it is inferred that the antifeeding effects of plant extracts can lead to slower growth of insect herbivores by reducing the insect growth parameters, which was also evident in our results. The botanicals alone and neem leaf extract in combination with pyriproxyfen caused significant negative effects on the food consumption and growth of the cabbage white butterfly larvae. So, certain plant part extracts can be used as insect repellents, antifeedants and growth inhibitors under certain conditions to produce toxin free food products.

However, the combination of pyriproxyfen with 7% neem extract showed lower mortality as compared to the application of 7% neem extract alone. Also, there was no significant difference in mortalities of *P. brassicae* larvae with the different ratios (1:2 and 2:1) of insecticide and botanical. The lack of synergistic effect might be due to detoxification mechanisms leading to inactivation of some metabolites. In this context, it is possible that the toxicity of pyriproxyfen and neem extract was reduced because the combination caused degradation of one or more constituents in the mixture (Yi et al., 2012).

It is also important that botanicals and pyriproxyfen have different mechanisms of action. For instance, pyriproxyfen is a juvenoid which disturbs the insect growth and causes mortality at a younger age (Ohba et al., 2013) and differential toxicities of insecticides depend on larval age, concentrations and exposure periods (Yue et al., 2003). While, neem is an antifeedant and toxicant for many insects, slowly disrupting insect growth (Morgan, 2009). The main mode of action of chemicals determines the sensitivity of exposed organisms (Sánchez-Bayo, 2012). However, botanical and insecticide synergism is important for control of insect pests. Efficacies of mixtures will depend on the insect species, strain and as well as the concentration ratio (Taillebois & Thany, 2016).

The larvae achieve faster growth when their food consumption and efficiency of conversion of ingested food is high. However, food ingestion, and its assimilation and conversion into energy, differs in accordance with the quality of the food. Feeding and growth indices are directly linked with the quality of food supplied. In this experiment, increased concentrations of plant extract greatly decreased RGR, RCR, and ECI parameters of the cabbage caterpillars. In fact, plant extract application led to reduced food quality with decreased the tendency of insects to consume food resulting in a lower growth rate. With the application of botanicals, the consumption of food can be reduced to decrease herbivore fitness. This feeding and growth relationship with food treated with botanicals can be utilized to achieve improved crop production.

Conclusions

Useful insecticidal and repellent properties of neem leaf extracts have been shown in the study, which indicates it can be used to suppress the cabbage white butterfly caterpillars in food crops. In addition, insecticide-botanical combinations can provide cost effective solutions for crop problems and can be used as an important part of integrated pest management strategies. However, achieving more acute interactions, the synergistic mechanism between insecticides and the botanicals should be the focus in future research.

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