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Research Article

Laboratory Evaluation of Selected Differential Chemistry and Botanical Insecticides against Hadda Beetle *Epilachna vigintioctopunctata* Fabricius (Coleoptera: Coccinellidae)

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Authors' Contributions
MUH performed experiments and recorded data. ABMR conceived and designed the experimental protocols and supervised the research. MZM did statistical analyses and prepared the results. MA prepared the initial draft of the manuscript. MIU provided technical assistance and proofread the final manuscript.

Keywords
Hadda beetle, Biorational insecticides, Botanical extracts, Binary combinations, *Azadirachta indica*, Spinosad

Abstract | Hadda beetle, *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae), is a cosmopolitan phytophagous pest and causes severe damage to various cucurbitaceous and solanaceous vegetables. This laboratory work was aimed to determine the comparative toxicity of three differential-chemistry insecticides (*i.e.* emamectin benzoate 1.9 EC, spinosad 240 EC and lufenuron 50 EC) and the aqueous extracts of three local plant species (*i.e. Eucalyptus camaldulensis* Dehn., *Citrus limon* L. and *Azadirachta indica* A. Juss) against the grubs and adults of *E. vigintioctopunctata.* Three concentrations of each synthetic insecticide (*i.e.* 2.5, 2 and 1.5%) and botanical extract (*i.e.* 10, 7.5 and 5%) were bioassayed using standard leaf-dip method. Results revealed that spinosad and 10% *A. indica* aqueous extract exhibited maximum mean cumulative mortality of hadda beetle grubs (*i.e.* 84.1 and 73.4%, respectively) and adults (*i.e.* 67.4 and 58.8%, respectively) recorded at 5th day of exposure. Combined application of both these effective treatments enhanced their toxicity to *E. vigintioctopunctata* and caused 88.9 and 77.6% cumulative mortality of grubs and adults, respectively. Based on overall study results, the combined application of spinosad and *A. indica* extract is recommended to local vegetable growers for effective integrated management of *E. vigintioctopunctata* and other foliage-feeding beetles.

Novelty Statement | This study demonstrates the synergistic action of the binary combinations of biorational synthetic insecticide (spinosad) and botanical extract (*Azadirachta indica*) which can be effectively used against the infestations of hadda beetle *Epilachna vigintioctopunctata* and other foliage beetles.

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Introduction

Hadda beetle, *Epilachna vigintioctopunctata* Fab. (Coleoptera: Coccinellidae), is among the destructive pests of various horticultural crops and can cause serious economic losses. This pest is widely distributed throughout the world including China, America, Malaya, India, Pakistan, Siberia, Sri Lanka and Australia (Jamwal *et al.*, 2013). *E. vigintioctopunctata* commonly feeds on solanaceous and cucurbitaceous plants in various regions worldwide (Anam *et al.*, 2006; Rahaman *et al.*, 2008). Both adults and grubs voraciously feed on the leaves of plants and affect the quality and quantity of crop plants by retarding their growth and ultimately reduce the production (Ali

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and Rizvi, 2007). Moreover, being polyphagous in nature, *E. vigintioctopunctata* may attack other vegetable crops such as brinjal, potato and tomato. In case of severe infestations, it scrapes the chlorophyll content of leaves and skeletonizes the leaves causing a papery structure on the infested leaves, which ultimately leads to considerable yield reduction (Islam *et al*., 2011).

Farmers in Pakistan predominantly rely on the extensive and frequent applications of conventional synthetic insecticides to control hadda beetle and other insect pests. Unfortunately, the continuous application of these persistent and toxic chemicals is causing various environmental problems such as residual effects in the environment (Aktar *et al*., 2009; Bolzonella *et al*., 2019), eradication of beneficial fauna and human health hazards (Koureas *et al*., 2012; Gontijo *et al*., 2014; Nicolopoulou-Stamati *et al*., 2016; Braak *et al*., 2018; Zhang *et al*., 2018). Furthermore, the extensive use of conventional synthetic chemicals is causing resistance development in insect pests including *E. vigintioctopunctata* (Kumar and Kumar, 1997; Hawkins *et al*., 2019).

Keeping in view the aforementioned ecological consequences of conventional synthetic insecticides, there is a need to find more effective alternatives that can control the pests with less environmental impact (Furlan and Kreutzweiser, 2015). For instance, there are some synthetic insecticides presently available in the markets which have a chemistry and mode of action different than the conventional ones. These softer insecticides have certain advantages over conventional insecticides such as they are highly selective to target pests, are effective at low dosage, and are relatively safer to natural enemies present in the field and for the environment as well (Kodandaram *et al*., 2010). Therefore, these differential chemistry insecticides are being considered relatively safe and fit well into the integrated pest management (IPM) programs against various insect pests.

Similarly, plant based pesticides such as botanical extracts and essential oils have long been suggested as attractive alternatives to synthetic chemicals for controlling insect pests (Miresmailli and Isman, 2014; Majeed *et al*., 2018, 2020). Botanicals are usually eco-friendly, cost-effective, easily biodegradable and target-specific. The main advantages of botanicals are their specificity and safety to humans (Miresmailli and Isman, 2014; Stevenson *et al*., 2017). These are cheaper, affordable and easy to prepare which are imperative qualities of pest control products for smallholder farmers (Stevenson *et al*., 2017).

Moreover, to reduce the non-target effects of synthetic pesticides on human health and environment, synthetic insecticides can be combined with other biorational control options such as botanicals (Obeng-Ofori and Amiteye, 2005; Athanassiou *et al*., 2009; Miresmailli and Isman, 2014). Combined application of different control tactics would be eco-friendly and more effective to suppress the pest populations (Barčić *et al*., 2006). In this laboratory study, some promising botanical extracts and some selected differential-chemistry insecticides alone and in combination were evaluated against the grubs and adults of *E. vigintioctopunctata*.

Materials and Methods

This laboratory work was performed in the Department of Entomology, College of Agriculture, University of Sargodha.

Insect rearing

Adult *E. vigintioctopunctata* beetles were collected from different fields of cucurbit and solanaceous vegetables situated in the premises of the College of Agriculture. The leaves along with beetles were cut-off with the help of a scissor and were brought to laboratory in plastic jars. Adults hadda beetles were kept in a glass cage (20 × 20 × 30 cm). The insects were reared under controlled conditions at 25±2 °C temperature and 65±5% R.H. Fresh brinjal (*Solamum melongena* L., cultivar Nirala) leaves were placed in cages and 10% sugar solution was provided to adults. Eggs were picked from the rearing cages and were arranged on moist filter paper discs lined in 60 mm glass Petri-plates. Newly emerged grubs were shifted to clean plastic jars and soft and tender leaves were provided to them. The culture was maintained up to three generations and 3rd instar grubs were used in the bioassays.

Preparation of botanical extracts

Leaves of neem (*Azadirachta indica* L.), eucalyptus (*Eucalyptus camaldulensis* Dehn.), and lemon (*Citrus lemon* L.) were collected from the field around College of Agriculture and were brought to the laboratory. After washing with distilled water, leaves were air-dried at room temperature (27 °C) for three days; followed by oven-drying at 60°C for 24 h. Dried leaves were grounded to obtain fine powder by an electrical blender. The aqueous solution of botanicals was prepared by mixing 100 g of powder of each plant in 1.0 L of water. The solution in glass vials was placed in a shaker at 40°C for 12 h. Filtration was done by using common filter paper to get the maximum refined form of each botanical and the solvent was evaporated finally by using Soxhlet apparatus (DH.WHM-12393, Daihan Scientific, South Korea). The stock solution was transferred in glass vials and was kept in the refrigerator for further use. Three concentrations, *i.e.*, 5, 7.5 and 10%, of each botanical extract were prepared in distilled water to determine their effectiveness against the hadda beetle grubs and adults.

Synthetic insecticides

Three synthetic insecticides with differential chemistry and mode of action than the conventional ones, *i.e.* emamectin benzoate (Proclaim® 19 EC; Syngenta), spinosad (Tracer® 240 SC; Dow Agro Sciences) and lufenuron (Match® 50 EC; Syngenta), were procured from an authentic pesticides dealer from the grain market of district Sargodha. Three concentrations, *i.e.,* 2.5, 2 and 1.5%, of each insecticide were prepared using distilled water for their toxicity evaluation against hadda beetles.

Bioassays

Leaf-dip bioassay method was followed to test different concentrations of each botanical and differential-chemistry synthetic insecticide. Leaves of brinjal (*S. melongena*) were collected from the field and were washed with distilled water. Leaves were dried at room temperature (27°C), and then were soaked in specific concentrations of insecticidal treatments for 10 sec and were dried for 10 min. Treated leaves were placed in 60 mm glass Petri-plates. Five grubs were released in each Petri plate. Each concentration of tested chemicals was replicated five times and five grubs were exposed in each replication. The same bioassay was performed for adult stages. Mortality data of grubs and adults were recorded after 1, 3, and 5 days of treatment applications.

Combined bioassay of botanicals and insecticides

The most effective botanical and insecticidal treatments appeared in the previous bioassays were further evaluated for their combined impact against *E. vigintioctopunctata* grubs and adults. Binary mixtures of aqueous extract of *A. indica* and spinosad, being the most effective treatments, were prepared to test against *E. vigintioctopunctata.* Each concentration of spinosad (*i.e.* 1.5, 2, and 2.5%) was mixed with each concentration of *A. indica* extract (*i.e.* 5, 7 and 10%). In the control treatment, distilled water was used. Bioassay protocol was same as described above. Each treatment was replicated five times and five individuals (grubs or adults) were exposed in each replication.

Data analysis

Data regarding the mortality of *E. vigintioctopunctata* grubs and adults were subjected to Abbott's formula (Abbott, 1925) for correction prior to the statistical analysis. Data were analyzed by two-factor analysis of variance (ANOVA) by keeping insecticides or botanicals and concentrations as main factors. Treatment means were separated by using LSD post-hoc test at a 5% probability level. All the analyses were performed by using SPSS® V. 20.0 software.

Results

There was a significant difference ($P < 0.05$) in percent mortality of *E. vigintioctopunctata* grubs by the application of insecticides at different concentrations at 1st, 3rd and 5th day of exposure. By increasing the concentration of each insecticide, the mortality of grubs was also increased. However, the highest mortality (*i.e.* 40.2, 62.6 and 84.0) of grubs was found by the application of spinosad with 1.5, 2.0 and 2.5% concentration, respectively recorded on the 5th day of exposure. Similar mortality pattern was found in case of adults, where all the insecticides at different concentrations showed significant ($P < 0.05$) mortality of beetles. By the application of spinosad at 1.5%, about 50.2% of adults were dead, followed by 57.6% at 2.0% concentration and 67.4% at 2.5% concentration (Table 1).

Botanicals at different concentrations showed significantly different ($P < 0.05$) mortality of grubs and adults of *E. vigintioctopunctata* at all observation days. The highest mortality of grubs (73.4%) was found after the application of 10.0% *A. indica* extract at the 5th day of exposure. Mortality of grubs was less than 45% by using *E. camaldulensis* and *C. lemon* plant extracts. Also, in the case of adults, *A. indica* exhibited higher mortality *i.e.* 58.8% of adults at 10.0% and 54.8% at 7.5% concentrations (Table 2).

Table 1: Mean corrected mortality (± SE) of grubs and adults of *Epilachna vigintioctopunctata* after the application of some selected differential-chemistry synthetic insecticides.

Treatments	Conc. (%)	Mean mortality (%) of grubs			Mean mortality (%) of adults		
		1 DAT	3 DAT	5 DAT	1 DAT	3 DAT	5 DAT
Lufenuron	1.5	13.4±0.90d	25.0±0.78d	35.8±0.93e	12.4±0.87c	16.6±0.58d	23.0±0.47d
	2.0	14.2±0.93d	33.8±0.92bcd	44.8±1.38cd	17.6±0.68c	19.6±0.62d	23.0±0.47d
	2.5	13.2±0.89d	31.4±0.88bcd	51.2±1.48c	19.0±0.68c	24.0±0.58d	28.4±0.28d
Emamectin benzoate	1.5	14.0±0.58d	27.6±0.67cd	37.6±1.39de	14.0±0.53c	21.2±0.38d	27.0±0.38d
	2.0	15.0±0.46cd	35.2±0.73bc	43.6±1.93cde	29.2±0.68b	34.8±0.48c	43.4±0.76c
	2.5	17.4±0.67cd	38.6±0.78b	51.4±1.43c	17.4±0.47c	33.4±0.27c	41.4±1.73c
Spinosad	1.5	22.0±0.83bc	27.2±0.58cd	40.2±1.84de	26.6±0.67b	38.0±0.46bc	50.2±1.48bc
	2.0	28.4±0.92b	38.2±0.73b	62.6±1.83b	39.8±0.82a	44.2±0.78b	57.6±1.48ab
	2.5	38.0±0.27a	52.4±1.38a	84.0±1.78a	44.6±0.89a	57.2±1.47a	67.4±1.39a
Significance		F = 2.69 P = 0.0463	F = 2.67 P = 0.0484	F = 8.21 P < 0.001	F = 5.09 P = 0.0024	F = 2.70 P = 0.0458	F = 4.58 P = 0.0043

DAT, day after treatment. Means sharing similar letters within a column are not significantly different at $P > 0.05$.

Table 2: Mean corrected mortality (± SE) of grubs and adults of *Epilachna vigintioctopunctata* after the application of some selected aqueous botanical extracts.

Treatments	Conc. (%)	Mean mortality (%) of grubs			Mean mortality (%) of adults		
		1 DAT	3 DAT	5 DAT	1 DAT	3 DAT	5 DAT
Lemon	5.0	14.4±0.34c	19.0±0.19e	30.2±0.47d	14.4±0.38ef	22.2±0.37cd	25.2±0.37c
	7.5	22.6±0.42b	23.4±0.37de	29.8±0.43d	14.0±0.37f	18.4±0.27d	24.0±0.38c
	10.0	24.0±0.25b	28.2±0.37c	33.0±0.37cd	18.8±0.27b-e	22.2±0.63cd	25.8±0.36c
Eucalyptus	5.0	16.2±0.26c	20.6±0.73e	32.6±0.36cd	16.0±0.54c-f	24.6±0.37cd	25.6±0.52c
	7.5	24.2±0.18b	26.4±0.37cd	37.2±0.67bc	23.4±0.58b	27.2±0.36bc	34.0±0.63b
	10.0	24.8±0.19b	33.0±0.67b	40.6±0.37b	29.2±0.62a	32.6±0.27b	37.5±0.32b
Neem	5.0	15.8±0.28c	22.0±0.27de	36.4±0.76bc	15.4±0.76def	27.2±0.37bc	31.0±0.37bc
	7.5	24.2±0.27b	35.8±0.47b	41.4±0.68b	19.8±0.67bcd	44.4±0.87a	54.8±1.37a
	10.0	35.6±0.42a	45.0±0.56a	73.4±1.38a	20.0±0.67bc	49.2±0.76a	58.8±1.49a
Significance		F = 3.05 P = 0.0292	F = 5.68 p = 0.0012	F = 23.84 P < 0.001	F = 3.03 P = 0.0297	F = 7.70 P = 0.0001	F = 8.77 P < 0.001

DAT, day after treatment. Means sharing similar letters within a column are not significantly different at P > 0.05.

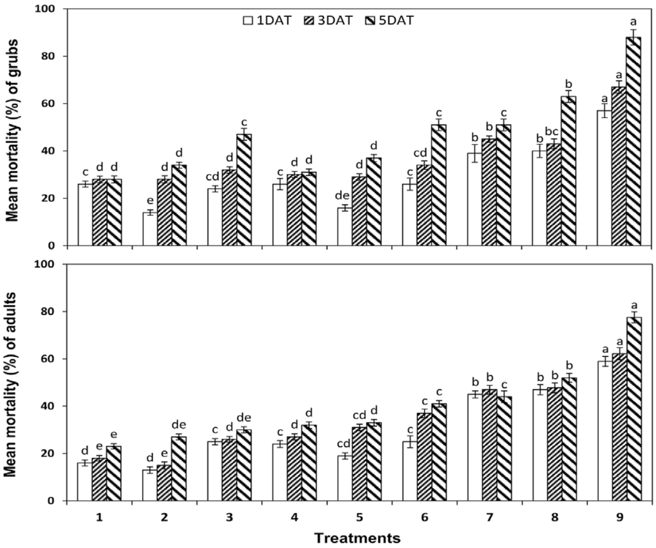

Figure 1: Mean corrected mortality (%) of grubs and adults of *Epilachna vigintioctopunctata* after combined application of spinosad and *Azadirachta indica* aqueous extract.

1: spinosad 1.5% + *A. indica* 5%; 2: spinosad 1.5% + *A. indica* 7.5%; 3: spinosad 1.5% + *A. indica* 10%; 4: spinosad 2% + *A. indica* 5%; 5: spinosad 2% + *A. indica* 7.5%; 6: spinosad 2% + *A. indica* 10%; 7: spinosad 2.5% + *A. indica* 5%; 8: spinosad 2.5% + *A. indica* 7.5%; 9: spinosad 2.5% + *A. indica* 10%. Means sharing similar letters for each time interval are not significantly different at P > 0.05.

A significant ($P < 0.001$) difference was found in the mortality of grubs and adults with a combined application of spinosad and *A. indica*. With increasing concentration of both insecticide and botanical, mortality of *E. vigintioctopunctata* was also increased. A mixture of higher concentrations of spinosad (2.5%) and *A. indica* (10.0%) showed 57.0% mortality of grubs at 1st day of exposure, followed by 67.0% on the 3rd day and 88.0% on the 5th day (Figure 1). Similar pattern of mortality response was observed in case of beetle adults. The highest mortality of adults (*i.e.* 59.0, 62.2 and 77.6% at 1st, 3rd and 5th day, respectively) was found for the binary combination of 2.5% spinosad and 10.0% *A. indica* extract (Figure 1).

Discussion

The use of synthetic insecticides is still the most effective strategy to control insect pests. However, the use of safer products for humans and the environment is a major concern. In this study, some differential-chemistry insecticides and local plant extracts were tested alone and in combination against grubs and adults of hadda beetle *E. vigintioctopunctata* which is a key pest of solanaceous and cucurbitaceous crops (Islam *et al.*, 2011) and management of this pest is still understudied. Limited information is available regarding the susceptibility of *E. vigintioctopunctata* to combined application of insecticides and botanicals.

Our bioassay results showed a significant mortality of *E. vigintioctopunctata* grubs and adults by spinosad alone at 2.5% concentration. Spinosad is a microbial insecticide derived from bacteria *Saccharopolyspora spinosa* and has been effective against a wide array of insect pests (Elliot *et al.*, 2007; Jha *et al.*, 2014; Reddy *et al.*, 2016; Shrestha *et al.*, 2020). It has been reported effective against alfalfa weevil *Hypera postica* (Reddy *et al.*, 2016), Colorado potato beetle *Leptinotarsa decemlineata* (Igrc *et al.*, 1999), cereal leaf beetle *Oulema melanoplus* (Buntin *et al.*, 2004), crucifer flea beetle *Phyllotreta cruciferae* (Elliot *et al.*, 2007) as well as against many stored product beetles (Fang *et al.*, 2002; Daglish and Nayak, 2006; Vayias *et al.*, 2009; Dissanayaka *et al.*, 2020). Moreover, our findings are consistent with the previous studies showing the effectiveness of spinosad against other foliage beetles. For instance, about 67 to 84% mortality of *E. vigintioctopunctata* grubs and adults was exhibited by spinosad (Buntin *et al.*, 2004; Elliot *et al.*, 2007).

Though synthetic insecticides are effective to manage insect pests, the increased risk of these chemicals to human health and environment, and the development of insecticide resistance in insect pests are contemporary concerns (Daglish and Nayak, 2006; Abrahams *et al*., 2017). Among the aqueous botanical extracts tested in this study, *A. indica* exhibited maximum mortality as compared to other extracts. This plant belongs to the family Meliaceae and exhibit multifarious anti-insect properties (Isman, 2002; Koul and Wahab, 2004). For instance, azadirachtin is the major constituent of neem extracts and has been reported effective against a wide array of insect pests (Brotodjojo and Arbiwati, 2016; Chaudhary *et al*., 2017) by deterring insect feeding and ovipositioning (Isman, 2002), inhibiting their digestive enzymes and disrupting molting hormones (Nisbet *et al*., 1996). *A. indica* products have been demonstrated very effective against other foliage beetles including *Henosepilachna vigintioctopunctata* and *E. vigintioctopunctata* (Murugesan and Murugesh, 2008; Mondal and Ghatak, 2009). *A. indica* is locally grown in many parts of Pakistan and would be recommended to local small-scale farmers as an effective alternate of synthetic pesticides.

Mixing synthetic insecticides with plant extracts could be a way to reduce the risk to humans and environment and to minimize the development of insect resistance. Combined action of insecticides may give more complete control than the use of a single insecticide (Athanassiou *et al*., 2008; Chintzoglou *et al*., 2008). Furthermore, the combinations of synthetic insecticides with other safe control agents like botanicals allow for a reduction in the concentration of insecticides.

Our findings showed that when spinosad was mixed with different concentrations of *A. indica* extract, the mortality of *E. vigintioctopunctata* increased synergistically, particularly in case of adult beetles. The combined action of spinosad and *A. indica* plant extract was described as independent synergism. For instance, Barčić *et al*. (2006) reported that the applications of spinosad along with neem (*A. indica*; Celaflor®) resulted in about 97% reduction of *L. decemlineata* larvae. The synergistic action of synthetic insecticides and botanicals could be due to the inhibition of insecticides detoxifying enzymes inside the insect body which consequently reduces lethal dose and toxicity of insecticides (Faraone *et al*., 2015).

Conclusions and Recommendations

Overall, the study results showed that spinosad among synthetic insecticides and *A. indica* among botanicals were effective treatments to control *E. vigintioctopunctata* grubs and adults. The combinations of these chemicals would be better than using alone for controlling *E. vigintioctopunctata* with respect to mortality of grubs and adults. Further study should be conducted for the combinations of spinosad with other chemicals including plant essential oils and entomopathogens that are suitable biorational control options against *E. vigintioctopunctata* and should be tested under the field conditions as demonstrated by Rahaman *et al*. (2008) and Javed *et al*. (2018).

Conflict of interest

The authors have declared no conflict of interest.

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