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SYNERGISM OF SELECTED PLANT ESSENTIAL OILS WITH ALPHA CYPERMETHRIN AGAINST *BACTROCERA ZONATA* (TEPHRITIDAE: DIPTERA)

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ABSTRACT

Current studies were carried out to evaluate the synergistic effect of selected essential oils when combined or mixed with alpha cypermethrin to reduce the insecticide dose as well increase the mixture efficacy in controlling adult *Bactrocera zonata*. Essential oil tested included *Parthenium hysterophorus* (PH), *Eucalyptus obliqua* (EO), *Cannabis sativa* (CS) each one used at five different concentrations selected as multiple (0.25, 0.5, 1, 2, 4) of their LC 50 values determined on the basis of preliminary trials. Constant ratio combination design was used for mixing the individual components. Different component mixtures included combinations of (PH + CS), (PH+EO), (EO+CS), (PH+CS+EO) each mixed at five concentration levels (same levels). Similarly, each of the essential oil was mixed with alpha Cypermethrin in combinations of multiple (0.25, 0.5, 1, 2, 4) of their LC 50 values. Toxicity of individual components as well as mixtures was determined by using dry film residue method. The response to Toxicity response exposure was estimated using the median-effect equation, as described by Chou and Talalay (1984). Interactions of these mixtures were studied using the combination-index (CI) equation method using the software Compusyn that is based on general equation of dose and effect and its theorem of combination index developed by using the approach of merging the physicochemical principle of the mass-action law with the mathematical principle of induction and deduction. LC values of individual essential oils as determined by the model were 0.55, 0.398 and 0.572 for *C. sativa*, *P. hysterophorus* and *E. obliqua* respectively. Cypermethrin exhibited LC50 value of 0.00353. When binary combination of cypermethrin and *c. sativa* were tested, the value of CI ranged between 0.53-1.74. (<1 being synergism) was observed only for the mixture combining 0.25(LC50) but the effect size was small (fa=0.6). All the binary combinations of cypermethrin alpha and *P. hysterophorus* showed antagonistic effect only. Cypermethrin when combined with *E. obliqua* indicated synergism at 0.25(LC50) and 4(LC50) with effect size of 0.68% and 0.9% respectively. In combination mixing all the three Eos with pyrethroids showed synergism only at highest concentration 4(LC50) with a very high effect size (fa=0.99). When Eos were mixed to see their synergistic effect, combination of *P. hysterophorus* with *E. obliqua*. Showed a moderate synergism (CI= 0.77) at the lowest concentration tested 0.25 (LC50) but the effect size was relatively smaller (fa=0.52). All the combination of *C. sativa* and *E. obliqua* showed antagonism (CI=1.26-2.09). In a binary combination of *P. hysterophorus* and *C. sativa*, a high level of synergism was observed at the highest concentration combination 4(LC50) with a very high effect size (fa=0.99). When all the three Eos were combined, Synergism was observed at 2(LC50) and 4(LC50) combinations with a high affect size (fa=>0.98).

Keywords: *Parthenium hysterophorus*, *Eucalyptus obliqua*, *Cannabis sativa*, alpha cypermethrin, Adult *Bactrocera zonata*, Acetone.

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INTRODUCTION

Fruit flies (*Diptera: Tephritidae*) are dominant species of pests all over the world and attack deteriorate a wide range

of fleshy fruits and vegetables in tropical and subtropical areas (Vargas et al., 2015). Some species have host specific feeding habits while other species are cosmopolitan and

infect a large variety of vegetables and fruits (Kapoor et al., 1980). One of the most noxious and injurious species of fruit flies are peach fruit fly (*Bactrocera zonata*), oriental fruit fly (*Bactrocera dorsalis*) and melon fruit fly (*Bactrocera cucurbitae*). These species are native to tropical Asia and widespread as far as Pakistan. *B. zonata* is native to South and Southeast Asia where it attacks more than 50 host plants, including peach, citrus, apricot, mango, guava and fig (White and Elson-Harris, 1992). The oriental fruit fly causes 5-100 percent losses to different fruits in Pakistan (Syed et al., 1970). About 80 percent damage in guava due to fruit flies is reported (Kafi, 1986). Kapoor (1993) also reported that fruit flies could cause 90 to 100 percent damage to ber fruits. In Pakistan about 24% losses were recorded in cucurbits due to *B. zonata*. About 50 to 80% infestation has been recorded in peach, pear, fig, apricot and other fruits, (Weems et al., 2010). A variety of different methods are employed for the control of fruit flies including, Sterile insect control, baits, cultural control, biological control, and use of botanical insecticides and cover sprays of conventional insecticides (Blomefield et al., 2015). Sterile insect technique in integration with other technique has been shown as an effective method for controlling fruit flies in South African context (Barnes et al., 2007). Bait application technique (BAT) in the form of bait stations or sprays (Barnes, 2000) is a common method of fruit flies' control. BAT in orchards is applied in the form of bait station (Ware et al., 2003). Bait sprays are usually applied several times throughout the season, with the number of applications depending on location, number of traps. Bait sprays take advantage of the fruit fly's attraction to protein, an important part of the bait. The flies feed on the insecticide in the bait and die (Broumas et al., 2002).

Among various culture control measures of fruit flies, Proper field cleanliness is important including removal of infested fruits (Plantwise, 2013). Grové et al. (2015) recommended the removal of any alternate host plants that appear in fruit production areas as they act as a breeding source and ground of the fruit flies. Biological control through different parasitoids is another environmentally friendly and safe method for pest management, which is concerned with releases of the predictable parasitoids, *D. giffardi* and *P. vindemniae* can be used as effective biological control agents (Van et al., 2006). *D. giffardii* has been recorded in more than 20 countries in the world to control the Dipteran pests (Noyes, 2002). Phytochemicals have the potential to manage various insect pests. Extracts of plants and other secondary metabolites of plants are becoming increasingly popular as alternatives to synthetic

pesticides due to their effectiveness, safety and minimal effects on non-target organisms. Bioinsecticides also have little potential for development resistance by target pests. Essential oils of some aromatic plants have been recognized to have cytotoxic, antioxidant, antifungal, insecticidal, and antibacterial properties. Although essential oils are relatively environmentally safer, these are not able to provide complete insect control if used alone. Various plant essential oils have toxic effects on various insects. Combination of different active ingredients may have a synergistic effect against insect pests.

Full-coverage sprays containing organophosphate insecticides or pyrethroids is generally considered as an effective control strategy, but excessive use of synthetic insecticides has serious detrimental effects on the environment, human beings and insect pests. There is a need to devise control methods that can minimize the number of synthetic insecticides. If combining plant essential oils with synthetic insecticides can increase the efficacy of chemical pesticides even when used at lower doses, it led to an overall reduction in the dose synthetic chemicals. This study was designed to explore the potential of synergizing the effect of synthetic pyrethroids by combining it with plant essential oils. Various concentrations of pyrethroids and essential oils will be combined and tested against *B. zonata* for topical toxicity and repellence effect. Synergism rate will be determined, and the best combination will be selected based on synergistic effects. Dose reduction index will be calculated for various combinations to determine the combination which is most effective in reducing the concentration of pyrethroids required to cause an effective mortality of *B. zonata*.

MATERIALS AND METHODS

This experiment was conducted in the entomology laboratory at the University of Swabi, KP, Pakistan. A peach fruit fly (*Bactrocera zonata*) adult was used in this experiment which is received from the culture and kept in the laboratory at $28 \pm 2^\circ\text{C}$, $65 \pm 5\%$ comparative moisture and 14:10h light and dark.

Preparation of plant essential oil (EO)

Three species of plants i.e., *Parthenium hysterophorus*, *Eucalyptus obliqua*, and *Cannabis sativa* collected from district Swabi. Each plant was shade dried at ambient temperature for 5–15 days, ground to a rough powder which was purified following the protocol of (Champakaew et al., 2015). About 250-300g of ground plant powder was kept in an extraction column connected to distillation flask holding 1600 mL of distilled water. The distilled water was then

boiled to 100°C in an immersion heater in order to produce vapor that would pass through the plant ingredients. The distilled mixture of oil and water was allowed to settle down into 2 coatings within a time period of over three to five days with the EO on top of the water. This procedure was lastly followed by the releasing of water gradually in order for the EO to stay.

Adulticidal Bioassay of Eos

Essential oils (EO) of *C. sativa*, *P. hystrophorus* and *E. obliqua* each at five concentrations were tested for their toxicity against *B. zonata*. The concentrations were chosen based on preliminary trials conducted to find EC 50 for each essential oil. The Concentrations were set as EC 50 value as well as values that were 0.25, 0.5, 2, and 4 times the LC50 value of each EO. The solutions were prepared using acetone as solvent. Two ways combinations were designed mixing each essential oil in a diagonal constant ratio combination design proposed by Chou and Talalay (1984). Finally, all the three essential oils were mixed together to test the compatibility of all the chemicals used together.

The experiment was performed using dry residue film method, (Yuying et al., 2013). A solution of relevant concentration was poured in to a 250ml conical flask, capped by aluminum foil and rolled for duration of 10 sec to coat the flask internally with the Eos where the extra solution was cast-off. The flask was then air dried by placing on a rack upside until the acetone evaporates. Acetone alone was used as control. Fourteen adult fruit flies which were of 5-8 days were introduced into each flask and kept at photoperiod of 14:10h (L:D) and 28±2°C. Record of the Mortality was made after 24h. Each trial was directed in triplicate thrice. The response of *Bactrocera zonata* to exposure of toxic chemicals (alone and in combination) was estimated using the software Compusyn which calculates the median-effect equation, as described by Chou and Talalay (1984):

$$\frac{f_a}{f_u} = \left(\frac{D}{LC_{50}} \right)^m$$

Where f_a is the fraction affected and f_u is unaffected fraction ($f_a = 1 - f_u$). D represents the dose affecting a fraction f_a . LC_{50} is the dose causing 50% mortality in *B. Zonata*. m is the coefficient of the shape of the dose-effect curve ($m = 1$, $m > 1$, and $m < 1$ indicate hyperbolic, sigmoidal, and negative sigmoidal dose-effect curves, respectively). r (Coefficient of correlation) is used as a good of fit parameter for median effect equation. The quantification of synergism or antagonism for a combination of a series of chemicals is given by combination index (CI)

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} = \frac{(D)_1}{(D_m)_1 [f_a/(1 - f_a)]^{1/m_1}} + \frac{(D)_2}{(D_m)_2 [f_a/(1 - f_a)]^{1/m_2}}$$

$CI < 1$, $CI = 1$, and $CI > 1$ indicate synergism, concentration addition, and antagonism, respectively.

Adulticidal bioassay of α -cypermethrin

Cypermethrin was tested alone and in combination with essential oils of *C. sativa*, *P. hystrophorus* and *E. obliqua* each at five concentrations. The concentrations were chosen based on preliminary trials conducted to find EC 50 for Cyperthmihrin and those of the three essential oils. The Concentrations were set as EC 50 value as well as values that were 0.25, 0.5, 2, and 4 times the LC50 value of each chemical. Two ways combinations were designed mixing Cypermethrin with each essential oil in a diagonal constant ratio combination design proposed by Chou and Talalay (1984). The resulting mixtures are shown in Table 1. All the mixtures were prepared using acetone as solvent. The experiment was performed by the procedure of dry residue film method as mentioned above. The response of *Bactrocera zonata* to exposure of toxic chemicals (alone and in combination) was estimated using the software Compusyn. The combination index was also calculated as mentioned above.

Statistical Analysis

Data was analyzed using the software “Compusyn” to determine the dose effect response and compatibility index of various combinations used in this experiment.

RESULTS AND DISCUSSION

Combination of essential oils

Median effect dose ranged from 0.39 to 0.57 for the essential oils used in the experiment with *P. hystrophorus* showing the highest toxicity. A high value of r is indicative of a high good of fit for the model used (Table 2, 3). When essential oils were combined in two-way combinations, it yielded to responses ranging from strong synergism to antagonism ($CI = 0.1$ to 2.1). when *P. hystrophorus* and *E. obliqua* were mixed together at lower concentration, a moderate degree of synergism was observed ($CI = 0.77$) (Table 2) but at this combination a lower value of fractional inhibition (0.52) was observed so even though the combination had synergistic effect, it would not be of practical significance to use it as sole control strategy against *B. zonata*. The rest of combinations had higher values of CI thus indicating lower synergism or even

antagonism. At very high concentrations of 4x of their LC50, the two-way combination yielded a moderate synergism with a high value of fractional inhibition (Fa=0.98) but still a similar inhibition was observed for *P.*

hysterophorus alone. Thus, although the combination increased the toxicity as compared with *E. obliqua* alone, it does not seem to be of practical significance (reducing the concentrations of active ingredients used).

Table 1: Combinations used in the experiment.

Cypermethrin (%)	<i>C. sativa</i> + Cypermethrin (%)		<i>P. hysterophorus</i> + Cypermethrin (%)		<i>E. obliqua</i> + Cypermethrin (%)		<i>C. sativa</i> + <i>E. obliqua</i> + <i>P. hysterophorus</i> + Cypermethrin (%)			
0.00158	0.195	0.00158	0.00158	0.33	0.1675	0.00158	0.195	0.33	0.1675	0.00158
0.00315	0.39	0.00315	0.00315	0.45	0.335	0.00315	0.39	0.45	0.335	0.00315
0.0063	0.78	0.0063	0.0063	0.62	0.67	0.0063	0.78	0.62	0.67	0.0063
0.0126	1.56	0.0126	0.0126	0.79	1.34	0.0126	1.56	0.79	1.34	0.0126
0.0252	3.12	0.0252	0.0252	0.88	2.68	0.0252	3.12	0.88	2.68	0.0252

Table 2: Combination of *P. hysterophorus* with *E. obliqua* against *B. zonata*.

Concentration (%) <i>P. hysterophorus</i>	Inhibition (fa)	Parameters			CI	Conclusion
		m	Dm	r		
0.210	0.4	1.49 ± 0.34		0.39	0.92	
0.421	0.52					
0.842	0.55					
1.684	0.86					
3.368	0.98					
<i>E. obliqua</i>		0.99 ± 0.08		0.57	0.98	
0.1675	0.21					
0.335	0.43					
0.67	0.5					
1.34	0.69					
2.68	0.83					
0.210	0.167				0.77	Moderate synergism
0.421	0.335				0.88	slight synergism
0.842	0.67				1.63	Antagonism
1.684	1.34				0.70	synergism
3.368	2.68				0.71	Moderate synergism

Parameter m represents the shape of resulting dose-effect curve; Dm shows the median effect dose that signifies potency, (m = 1 is hyperbolic while m > 1, and m < 1 show sigmoidal and negative sigmoidal curves, respectively). r (Coefficient of correlation) is used as a good of fit parameter for median effect equation. CI is the Synergism Index and a value of CI lesser than 1 indicates synergism and a value greater than 1 show, CI=1 shows antagonism. A value of 1 means additive-effect.

When *C. sativa* and *E. obliqua* were mixed in a 2-way mixture, the resulting mixture showed moderate to high degree of antagonism (Table 3) except when mixed at the highest concentration (4x LC 50) the mixed showed a strong synergistic effect (CI=0.1) along with a very high value of fractional inhibition (0.99) which was higher than when both were used separately. When *C. sativa* and *P. hysterophorus* were mixed together in a 2-way combination,

the CI values ranged from 0.4 to 1.19 (synergism to additive effect) but high degree of fractional inhibition was observed only at very high concentrations (Table 4).

Table 5 shows a mixture of all the three essential oils. The values of CI ranged from 0.41 to 1.25 (synergism to moderate antagonism). Higher degrees of synergism were observed only when all the three essential oils were mixed at concentrations equal to higher than their LC50 values.

When mixed in ratios of 2x LC 50, a high degree of synergism was observed along with a high value of fractional inhibition (0.98). When the oils were mixed in ratios of LC50 value, the resulting mixture showed a slight synergism (CI=0.90) with a fractional inhibition of 88%

which is much better than any of the essential oils used individually at this concentration. It can be concluded that mixing all the three essential oils at their median level concentrations can be a desirable method of using it as a control tactic for *B. zonata*.

Table 3: Combination of *C. sativa* with *E. obliqua* against *B. zonata*.

Concentration (%)		Fractional Inhibition (fa)	Parameters			CI	Conclusion
<i>C. sativa</i>			m	Dm	r		
0.195		0.26	0.96 ± 0.18	0.54	0.95		
0.39		0.45					
0.78		0.52					
1.56		0.74					
3.12		0.86					
<i>E. obliqua</i>			0.99590 +/- 0.08440	0.57	0.98		
0.1675		0.21					
0.335		0.43					
0.67		0.5					
1.34		0.69					
2.68		0.83					
0.195	0.1675	0.33				1.44	Moderate Antagonism
0.39	0.335	0.4				2.11	Antagonism
0.78	0.67	0.57				2.09	Antagonism
1.56	1.34	0.81				1.26	Moderate antagonism
3.12	2.68	0.99				0.10	strong synergism

Parameter m represents the shape of resulting dose-effect curve; Dm shows the median effect dose that signifies potency, (m = 1 is hyperbolic while m > 1, and m < 1 show sigmoidal and negative sigmoidal curves, respectively). r (Coefficient of correlation) is used as a good of fit parameter for median effect equation. CI is the Synergism Index and a value of CI lesser than 1 indicates synergism and a value greater than 1 show, CI=1 shows antagonism. A value of 1 means additive-effect.

Table 4: Combination of *C. sativa* with *P. hysterophorus* against *B. zonata*.

Concentration (%)		Fractional Inhibition (fa)	Parameters			CI	Conclusion
<i>C. sativa</i>			m	Dm	r		
0.195		0.26	1.0 ± 0.08278	0.55	0.98		
0.39		0.45					
0.78		0.52					
1.56		0.74					
3.12		0.86					
<i>P. hysterophorus</i>			1.49 ± 0.34	0.39	0.92		
0.2105	0.4						
0.421	0.52						
0.842	0.55						
1.684	0.86						

	3.368	0.98		
0.195	0.2105	0.5	0.87	Slight synergism
0.39	0.421	0.62	1.19	slight antagonism
0.78	0.842	0.83	1.01	Nearly additive
1.56	1.684	0.93	0.95	Nearly additive
3.12	3.368	0.99	0.44	synergism

Parameter m represents the shape of resulting dose-effect curve; Dm shows the median effect dose that signifies potency, ($m = 1$ is hyperbolic while $m > 1$, and $m < 1$ show sigmoidal and negative sigmoidal curves, respectively). r (Coefficient of correlation) is used as a good of fit parameter for median effect equation. CI is the Synergism Index and a value of CI lesser than 1 indicates synergism and a value greater than 1 show, CI=1 shows antagonism. A value of 1 means additive-effect.

Table 5: Combination of *C. sativa*, *P. hystrophorus* and *E. Obliqua* against *B. zonata*.

Concentration (%) <i>C. Sativa</i>	Fractional Inhibition (fa)	Parameters			CI	Conclusion
		m	Dm	r		
0.195	0.26	1.005 $\pm$ 0.08	0.55	0.98		
0.39	0.45					
0.78	0.52					
1.56	0.74					
3.12	0.86					
<i>P.</i>						
<i>hystrophorus</i>						
0.2105	0.4	1.49 $\pm$ 0.34	0.398	0.92		
0.421	0.52					
0.842	0.55					
1.684	0.86					
3.368	0.98					
<i>E.Obliqua</i>						
0.1675	0.21	0.99 $\pm$ 0.08	0.57	0.98		
0.335	0.43					
0.67	0.5					
1.34	0.69					
2.68	0.83					
0.195	0.2105	0.167	0.48		1.25	Moderate antagonism
0.39	0.421	0.33	0.71		1.10	Slight antagonism
0.78	0.842	0.67	0.88		0.90	Slight synergism
1.56	1.684	1.34	0.98		0.41	Synergism
3.12	3.368	2.68	0.99		0.49	Synergism

Parameter m represents the shape of resulting dose-effect curve; Dm shows the median effect dose that signifies potency, ($m = 1$ is hyperbolic while $m > 1$, and $m < 1$ show sigmoidal and negative sigmoidal curves, respectively). r (Coefficient of correlation) is used as a good of fit parameter for median effect equation. CI is the Synergism Index and a value of CI lesser than 1 indicates synergism and a value greater than 1 show, CI=1 shows antagonism. A value of 1 means additive-effect.

Combining essential oils with cypermethrin

C. sativa and Cypermethrin

Cypermethrin used alone exhibited fractional inhibition in the range of 0.33 to 0.88 for the minimum and maximum concentrations used with a median effect of 0.0035% while

C. sativa alone showed a fractional inhibition of 0.88 when used at highest concentration. When mixed at lowest concentrations (0.25x LC50), a highly synergistic effect (CI=0.54) was observed but with a low fractional inhibition (60%) (Table 6). The synergistic effect reduced with

increasing concentrations of the mixture thus mixing *C. sativa*: cypermethrin at 0.39:0.00315 (0.5x Lc50) a slight synergism was observed but with still with a low fractional inhibition rate (64%). Further increase in concentrations of the mixture led to additive effect (CI=1.04) and then antagonism (CI=1.74) at higher concentrations. This indicates that *C. sativa* and cypermethrin when mixed at

lower concentration have synergistic effect but with a lower fractional inhibition. At higher concentration although the fractional inhibition is higher, the mixture shows more of antagonistic effect. Thus mixing *C. sativa* with cypermethrin does not seem to be a viable option for reducing the concentration of cypermethrin.

Table 6: Combination of *C. sativa* with alpha cypermethrin against *B. zonata*.

Concentration (%) <i>C. sativa</i>	Inhibition (fa)	Parameters			CI	Conclusion
		m	Dm	r		
0.195	0.26	1.0± 0.08	0.55	0.98		
0.39	0.45					
0.78	0.52					
1.56	0.74					
3.12	0.86					
cypermethrin						
0.00158	0.33	0.99±0.04	0.003	0.99		
0.00315	0.45					
0.0063	0.62					
0.0126	0.79					
0.0252	0.88					
0.195	0.00158				0.53	synergism
0.39	0.00315				0.89	slight synergism
0.78	0.0063				1.12	slight antagonism
1.56	0.0126				1.04	Nearly additive
3.12	0.0252				1.74	slight antagonism

Parameter m represents the shape of resulting dose-effect curve; Dm shows the median effect dose that signifies potency, ($m = 1$ is hyperbolic while $m > 1$, and $m < 1$ show sigmoidal and negative sigmoidal curves, respectively). r (Coefficient of correlation) is used as a good of fit parameter for median effect equation. CI is the Synergism Index and a value of CI lesser than 1 indicates synergism and a value greater than 1 show, CI=1 shows antagonism. A value of 1 means additive-effect.

***P. hysterophorus* and Cypermethrin**

Combination of *P. hysterophorus* with cypermethrin yielded an antagonistic response at the concentration used (Table 7) wherein high values of CI were observed (1.9 to 3.9). This mixture seems to be highly incompatible for use against *B. zonata*.

***E. obliqua* and Cypermethrin**

When *E. obliqua* was mixed with cypermethrin, a synergistic response was observed at lowest concentrations (Table 8) but with a low fractional inhibition (52%). With the increase in concentrations of both the ingredients, the mixture showed an increase in CI values indicating a shift from synergism to antagonism.

***C. sativa*, *P. hysterophorus*, *E. obliqua* and Cypermethrin**

When all the three essential oils were mixed with cypermethrin, the values of CI varied from 0.56 to 1.94 for different concentrations (Table 9). At lower concentrations, the mixture had higher CI values showing antagonism of all the four ingredients used while at higher concentrations synergism was observed. when mixed at 1.56:1.68:1.34:0.126 (% concentration) of *C. sativa*: *P. hysterophorus*: *E. obliqua*: cypermethrin. The mixture which was a combination of 2x LC50 values resulted in a high fractional inhibition (94%). Mixtures with the highest concentration had a higher fractional inhibition but with a relatively higher value of CI (0.56).

Table 7: Combination of *P. hysterophorus* with alpha cypermethrin against *B. zonata*.

Concentration (%) <i>P. hysterophorus</i>	Inhibition (fa)	Parameters			CI	Conclusion
		m	Dm	r		
0.2105	0.4	1.49 ± 0.34	0.39	0.92		
0.421	0.52					
0.842	0.55					
1.684	0.86					
3.368	0.98					
cypermethrin						
0.00158	0.33	0.99 ± 0.04	0.003	0.99		
0.00315	0.45					
0.0063	0.62					
0.0126	0.79					
0.0252	0.88					
0.2105	0.00158	0.24			2.56	Antagonism
0.421	0.00315	0.5			1.95	Antagonism
0.842	0.0063	0.5			3.90	strong antagonism
1.684	0.0126	0.81			2.43	Antagonism
3.368	0.0252	0.88			3.19	Strong antagonism

Parameter m represents the shape of resulting dose-effect curve; Dm shows the median effect dose that signifies potency, ($m = 1$ is hyperbolic while $m > 1$, and $m < 1$ show sigmoidal and negative sigmoidal curves, respectively). r (Coefficient of correlation) is used as a good of fit parameter for median effect equation. CI is the Synergism Index and a value of CI lesser than 1 indicates synergism and a value greater than 1 show, CI=1 shows antagonism. A value of 1 means additive-effect.

Table 8: Combination of *E. obliqua* with alpha cypermethrin against *B. zonata*.

Concentration (%) <i>E. obliqua</i>	Inhibition (fa)	Parameters			CI	Conclusion
		m	Dm	r		
0.167	0.21	0.99 ± 0.08	0.572	0.98		
0.335	0.43					
0.67	0.5					
1.34	0.69					
2.68	0.83					
cypermethrin						
0.0015	0.33	0.99 ± 0.04	0.003	0.99		
0.0031	0.45					
0.006	0.62					
0.0126	0.79					
0.0252	0.88					
0.167	0.0015	0.52			0.68	Synergism
0.335	0.0031	0.6			0.98	Nearly additive
0.67	0.006	0.71			1.20	Moderate antagonism
1.34	0.0126	0.9			0.65	Synergism
2.68	0.0252	0.92			1.30	Moderate antagonism

Parameter m represents the shape of resulting dose-effect curve; Dm shows the median effect dose that signifies potency, ($m = 1$ is hyperbolic while $m > 1$, and $m < 1$ show sigmoidal and negative sigmoidal curves, respectively). r (Coefficient of correlation) is used as a good of fit parameter for median effect equation. CI is the Synergism Index and a value of CI lesser than 1 indicates synergism and a value greater than 1 show, CI=1 shows antagonism. A value of 1 means additive-effect.

Table 9: Combination of *C. sativa*, *P. hystrophorus* and *E. Obliqua* with Alpha cypermethrin against *B. zonata*.

Concentration (%)				Fractional	Parameters			CI	Conclusion
<i>C. Sativa</i>				Inhibition (fa)	m	Dm	r		
0.195				0.26	1.00 ± 0.08	0.55	0.98		
0.39									
0.78									
1.56									
3.12									
<i>P. hystero</i> <i>phorus</i>									
0.2105				0.4	0.99 ±- 0.04	0.003	0.99		
0.421									
0.842									
1.684									
3.368									
<i>E. Obliqua</i>									
0.1675				0.21					
0.335									
0.67									
1.34									
2.68									
cypermethrin									
0.00158				0.33					
0.00315									
0.0063									
0.0126									
0.0252									
0.195	0.2105	0.1675	0.00158	0.45				1.93	Antagonism
0.39	0.421	0.335	0.00315	0.64				1.94	Antagonism
0.78	0.842	0.67	0.0063	0.81				1.82	Antagonism
1.56	1.684	1.34	0.0126	0.94				0.48	Synergism
3.12	3.368	2.68	0.0252	0.99				0.56	Synergism

Parameter m represents the shape of resulting dose-effect curve; Dm shows the median effect dose that signifies potency, ($m = 1$ is hyperbolic while $m > 1$, and $m < 1$ show sigmoidal and negative sigmoidal curves, respectively). r (Coefficient of correlation) is used as a good of fit parameter for median effect equation. CI is the Synergism Index and a value of CI lesser than 1 indicates synergism and a value greater than 1 show, CI=1 shows antagonism. A value of 1 means additive-effect.

CONCLUSION

The study was aimed to find mixtures that had the highest synergistic effect at lower concentrations so that we can increase the efficiency of essential oils as well as decrease the concentrations of cypermethrin needed to achieve an effective control of *B. zonata*. The combinations showed a whole range of activity from antagonism to synergism. Even if the mixtures show synergism and the mortality afflicted is not high enough (>80%), it will not be of practical significance. Similarly, if a high synergism is found along with high rates of mortality but the required concentrations are too high it may also be undesirable. We found out that

when all the essential oils viz *C. sativa*, *P. hystrophorus* and *E. obliqua* were mixed together in ratios of LC50 value, the resulting mixture showed a slight synergism (CI=0.90) with a fractional inhibition of 88% which is much better than any of the essential oils used individually at this concentration. It can be concluded that mixing all the three essential oils at their median level concentrations can be a desirable method of using it as a control tactic for *B. zonata*. When essential oils were mixed with cypermethrin When mixed at 2x LC50, although, the mixture resulted in a high fractional inhibition (94%), but it does not seem to be a desirable option as it requires mixing of cypermethrin at a

higher concentration. The study shows that although essential oils used alone may not be able to provide a satisfactory control of *B. zonata*, but their proper combinations can be used in a synergistic fashion to control *B. zonata*.

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