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Aptness of altered formulations of formic acid at different doses against *Varroa destructor,* a noxious ectoparasitic mite honeybee *Apis mellifera* ligustica

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Extensive use of acaricides like coumaphos, amitraz, flumethrin, and fluvalinate for the control of mites has led to the development of resistance and residues in bee hive products. Therefore, to overcome these situations, there is an urgent need to use natural compounds like organic acids. The present experiment was carried out at Honeybee Research Institute (HBRI), National Agricultural Research Centre (NARC), Islamabad to assess the efficacy of 60%, 65%, and 70% formulations of formic acid at three different doses i.e., 5ml, 10ml, and 15ml against *Varroa destructor*. The results showed that a 15ml dose of 65% formic acid caused the highest mean mortality of *V. destructor* (319 individuals) followed by 15ml of 70% formulation (249 individuals), 10ml of 65% formulation (238.33 individuals), and 15ml of 60% formulation (185.67 individuals).The highest efficacy against *V. destructor* was recorded at a 15ml dose of 65% formulation (84.31%) of formic acid followed by 10ml of 65% formulation (76.50%), 15ml of 70% formulation (63.34%), 5ml of 65% formulation (59.75%) and 10ml of 70% formulation (56.22%).It is concluded that the application of 65% formic acid with a dose of 15ml per colony is the most effective for the control of *V. destructor* mites in *Apis mellifera* colonies without any detrimental effects on the adult workers and their brood. Further studies should focus on the efficacy of formic acid and other organic acids or essential oils combinations on ectoparasitic mites.

Keywords: Formic acid, Varroa destructor, efficacy, mortality, organic acids

INTRODUCTION

Honeybees as the best pollinator play a key role in the ecosystem functioning and crop productivity. It has been found that about 35% of crop plants are pollinated by pollinators, especially honeybees (Klein et al . 2007; Garibaldi et al . 2013).Nowadays, it has been recorded that approximately 4,000 beekeepers in Pakistan are rearing 0.4 million colonies of Apis mellifera and producing ten thousand metric tons of honey each year which benefits 27,000 families (PARC, 2010-11). The colonies of A. mellifera are attacked by several pests like mites, greater wax moth, small hive beetle, hornets, and diseases like chalkbrood, foulbrood, Nosema, and viral diseases (Tan et al . 2007; Peña-Chora et al . 2023). The ectoparasitic mites, Varroa destructor is the single factor responsible for the declining of honeybee, Apis mellifera health worldwide and causing greater economic losses to apiculture industry in Pakistan (Noor et al. 2023).

Among them, one of the predominantly important pests is the Varroa destructor which is distributed throughout the world and is considered the major threat to apiculture (Peña-Chora et al . 2023).Apis mellifera and *A. cerana* are severely affected by *V. destructor* which causes severe damage to honeybee health and because of its social behavior, this mite widely transferred from the infected colony to other healthy colonies(Nekoei et al . 2023).Feeding by V. destructor may damage the different stages of honeybees such as weight, young one's lifecycle, immunity, and performance of adult workers (Ramsey et al . 2019). In Pakistan, Varroa mite became a serious pest of A. mellifera and damaged honeybee colonies in greater numbers (Ahmad, 1988; Sajid et al . 2020). Few studies have reported that V. destructor becomes a serious pest of honeybee colonies that causes severe economic loss to the apiculture (Anderson and Trueman, 2000). De Jong (1990) found that V. destructor can cause 100% mortality in A. mellifera colonies if left untreated for two consecutive years.

Various synthetic chemicals are used by beekeepers for the control of Varroa mite and extensive use of these chemicals cause resistance in mites. For the control of

this mite integrated-based approach should be adopted but sometimes did not show efficient results due to the complicated lifecycle of mites (Jack and Ellis, 2021).Honeybee colonies are weakened worldwide and have an alarming situation due to population decline. Researchers suggest that all the suitable methods for the control of mites should be adopted and those matricides' should be banned that cause resistance in mites as well as improvement in the hive hygienic technologies to reduce the diseases. Moreover, some insect pests of honeybees serve as a vector that transfer diseases resulting in the reduction of honey production and ultimately economy (Matthijs et al. 2020).

Various types of treatments are used by beekeepers for the control of *Varroa destructor*. Reliable controlling measures are still unknown that may decrease the population of this ectoparasite.To tackle this issue, different control measures including tartaric, lactic, formic, and citric acids can be applied (Vilarem et al . 2023).Vimla and Khan (2013) reported that formic acid causes 72.94% mortality in mites when used for up to three weeks. Petropolis and Formato (2022) found that the application of 60% formic acid concentration with a 290 ml dose showed higher mortality of *V. destructor* in treated colonies as compared to untreated colonies. Most of the work from Pakistan has been done on the application of different formulations of formic acid toreduce the population ofectoparasitic mites (Qadir*et al* . 2021; Mahmood et al . 2011; 2012; Islam et al . 2017). The literature regarding the use of different doses of formic acid formulations is scarce (Qadir et al. 2021).

Therefore, keeping in view the importance of honeybee mites, this experiment was designed to measure the efficacy of different formulations of formic acid at three different doses against *V. destructor* in *A. mellifera* colonies.

MATERIALS AND METHODS

Study area

The experiment was conducted from July to August at the apiary of Honey Bee Research Institute (HBRI) (33°40'31"N 73°07'34"E; 508 m above sea level), National Agricultural Research Centre (NARC), and Islamabad, Pakistan.

Colony selection

Thirty honeybee colonies naturally infested with *Varroa destructor* mites were selected for the experiment. week before treatments were applied, adults by sugar roll method (Gregorc et al . 2017) and sealed brood populations by opening 100 cells of worker brood (Mahmood et al . 2012)of test colonies were assessed for *V. destructor* infestation. In selected Langstroth hives, each bee colony had ten complete frames of worker bees and three to five brood frames. A bottom board tray consisting of a white for mica sheet under the mesh was placed in each hive for the collection of fallen mites (Islam et al. 2017). To restrict the entry of bees in the bottom board tray, wire screen was placed to cover the formica sheet (Calderone and Lin, 2003; Gregorc and Planinc, 2005). Ten treatment groups were randomly allocated to honeybee colonies. For each dose of formic acid formulations, three colonies were used. For control, three colonies were selected and remained untreated (Satta et al. 2005).

Chemical formulations and doses

Three formulations of formic acid with 85% Purity i.e., 60%, 65%, and 70% were used. Because this is locally available in the market and beekeepers are using it for the treatment. Three doses i.e., 5ml, 10ml, and 15ml for each formulation were applied to the selected honeybee colonies. The doses of each formulation were applied on the card of 2 × 2 inches with the help of a syringe (Mahmood et al . 2012).

Mortality of *Varroa destructor*

On a weekly basis, the total individuals of fallen dead mites/hive from treated and control colonies were counted (Floris et al. 2001; Gregorc and Planinc, 2005; Islam et al . 2017). The dead or fallen V. destructor were collected and counted under the microscope on the sampling dates. The formic sheets were placed again in the hive after cleaning with the help of a bee brush (Gregorc and Smodis Skirl,2007).

Knockdown effect:

At the end of the experiment, Fluvalinate (Apistan) strips were given to each experimental colony including control for knockdown. After 30 days the dead or fallen *V. destructor* was counted and Apistan strips were removed from the experimental colonies (Mahmood et al . 2017).The efficacy of the treatments was calculated for each colony using a given formula by Mahmood et al .(2012; 2017).

$$Efficacy\ (\%) = \frac{No.\ of\ mites\ fallen\ during\ treatment}{Total\ number\ of\ fallen\ mites (per\ and\ post\ treatment)} \times 100$$

Statistical analysis:

To compare different treatments, Analysis of variance (ANOVA) was applied with post-hoc test i.e., Least Significant Difference (LSD) at α 0.01.We used IBM-SPSS statistical software version 26 to apply ANOVA.

RESULTS

Different acaricides have various effects on *Varroa destructor* depending on the nature of acaricide formulation, mode of application, and weather conditions (Papežíková In the present study, the results of two-factor ANOVA showed that there was a statistically significant difference between the mortality of *V. destructor* in terms

of different formulations i.e., 60%, 65%, and 70% (F = 1151.77, df = 2, p-value = <0.0001), doses i.e., 5ml, 10ml and 15ml (F = 1974.30, df = 2, p-value = <0.0001) and formulation + doses (F = 63.90, df = 4, p-value = <0.0001) (table 1).

Table 1: Results of two-factor ANOVA on mortality of *Varroa destructor* at different formulations and doses of formic acid

Source	Sum of Squares	df	Mean Square	F	p-value
Corrected Model	122682.74[a]	8	15335.34	813.47	<0.0001
Intercept	932232.93	1	932232.93	49450.47	<0.0001
Formulation	43426.07	2	21713.04	1151.77	<0.0001
Dose	74438.30	2	37219.15	1974.30	<0.0001
Formulation * Dose	4818.37	4	1204.59	63.90	<0.0001
Error	339.33	18	18.85		
Total	1055255.00	27			
Corrected Total	123022.07	26			

a. R Squared = .997 (Adjusted R Squared = .996)

Table 2: Results of two-factor ANOVA on the efficacy of different formulations and doses of formic acid against *Varroa destructor*

Source	Sum of Squares	df	Mean Square	F	p-value
Corrected Model	6068.66[a]	8	758.58	386.34	<0.0001
Intercept	88890.80	1	88890.80	45271.61	<0.0001
Formulation	3864.98	2	1932.49	984.21	<0.0001
Dose	2170.42	2	1085.21	552.69	<0.0001
Formulation * Dose	33.26	4	8.32	4.24	0.014
Error	35.34	18	1.96		
Total	94994.80	27			
Corrected Total	6104.00	26			

a. R Squared = .994 (Adjusted R Squared = .992)

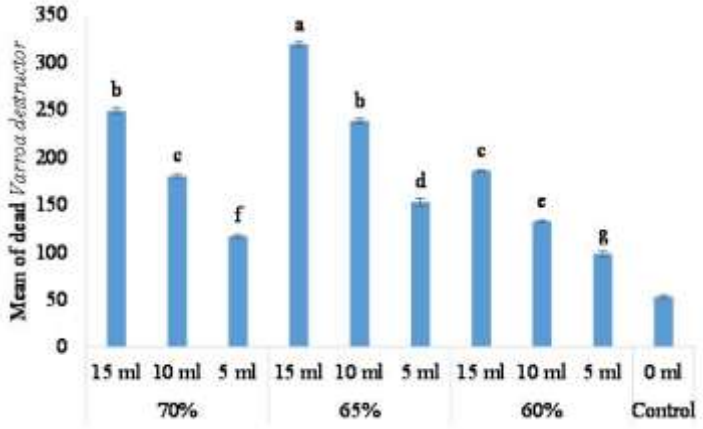

Figure 1: Mean mortality of *Varroa destructor* at different doses of different formulations

The highest mean mortality of*V. Destructor* was recorded at a 15ml dose of 65% formulation (319 individuals) of formic acid followed by 15ml of 70% formulation (249 individuals), 10ml of 65% formulation (238.33 individuals), 15ml of 60% formulation (185.67 individuals) and 10ml of 70% formulation (180 individuals). Whereas the lowest mean mortality was recorded at a 5ml dose of 60% formulation comprised of98.33 individuals of *V. destructor* (Fig. 1).In our results, the highest mean mortality of *V. destructor* was recorded at a 15ml dose of 65% formic acid. Samatarro et al . (2008) also reported the similar findings, who found that the application of 15ml formic acid (65%) caused 55 to 60% mite mortality in brood cells which can be increased from 87 to 89% in trapped worker brood (Calderone, 2000).

In the present study, the results of two-factor ANOVA showed that there was a statistically significant difference between the efficacy of different formulations i.e., 60%, 65%, and 70% (F = 984.21, df = 2, p-value = <0.0001), doses i.e., 5ml, 10ml and 15ml (F = 552.69, df = 2, p-value = <0.0001) and formulation + doses (F = 4.24, df = 4, p-value = 0.014) against *V. destructor*(table 2). The highest

efficacy against *V. destructor* was recorded at a 15ml dose of 65% formulation (84.31%) of formic acid followed by 10ml of 65% formulation (76.50%), 15ml of 70% formulation (63.34%), 5ml of 65% formulation (59.75%) and 10ml of 70% formulation (56.22%). Whereas the lowest mean efficacy against *V. destructor* was recorded at a 5ml dose of 60% formulation comprised of 34.92% (Fig. 2)

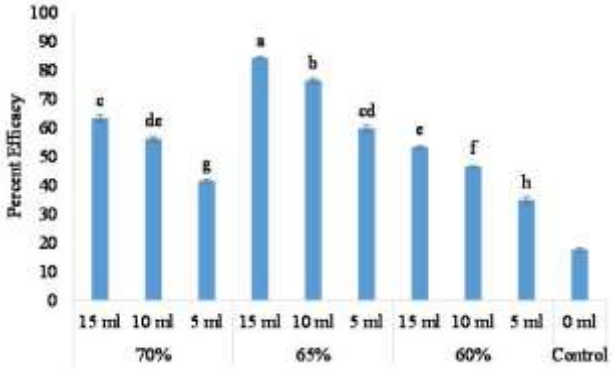

Figure 2: Percent efficacy of different formulations at different doses against *Varroa destructor*

Discussion

In our results, the highest efficacy against *V. destructor* was recorded at a 15ml dose of 65% formic acid. Contrarily, Qadir et al. (2021) found that treatment of 20ml formic acid (65%) dose exhibited more than 85% efficacy followed by 15ml and 10ml. These variations in the efficacy of formic acid might be due to several factors i.e., placement of formic acid plates inside the colony (Fries, 1989; Hoppe et al. 1989; Greatti et al . 1993), method of application (Ambrose et al . 2017), dose (Qadir et al . 2021), time of the year and temperature (Maul et al . 1980; Eischen, 1998; Skinner et al . 2001; Underwood and Currie, 2003).Previous studies have reported that different formulations of formic acid at different doses showed the highest efficacy against Varroa mites (Aziz et al . 2015; Pietropaoli and Formato, 2018; Pietropaoli and Formato, 2022).

Most of the literature has already reported that formic acid is quite effective in the control of ectoparasitic mites of honeybees. Mahmood et al. (2011) found that in winter season a 20ml dose of formic acid is most effective against V. destructor after the harvesting of sider honey. Islam et al. (2016) found that treatment of formic acid 65% provides the highest mortality (71.50%) in *V. destructor* with 323 individuals of dead Varroa mites falling on the sheet.Few studies reported that formic acid caused more than 90% mortality in *V. destructor* (Eguaras et al . 1996; Bahrein I et al . 2004; Abd El-Wahab and Ebada, 2006). The application of 65% formic acid twice at the interval of 10 days exhibited the lowest infestation levels of V. destructor than a single application of 65% formic acid (Căuia and Căuia, 2022).

CONCLUSIONS

It is concluded that the application of 65% formic acid with a dose of 15ml per colony is the most *effective for the control of V. destructor mites in* Apis mellifera colonies without any detrimental effect on the brood and adult bees.

Supplementary materials

Not applicable

Author contributions

Zamin Hussain Dahri, Waseem Akram, and Sumera Aslam conducted research, collected data, and manuscript write-up. Rashid Mahmood and Ghulam Sarwar conceived the idea. Muhammad Khalid Rafique contributed to data entry and statistical analysis and methodology.

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Institutional Review Board Statement

The study was approved by the Bioethical Committee of the Honeybee Research Institute, NARC, Islamabad.

Informed Consent Statement

Not Applicable.

Data Availability Statement

All of the data is included in the article/Supplementary Material.

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.
OR The authors declare no conflict of interest.

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