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EFFICACY OF MUSTARD STEMS POWDER AND ASH AGAINST *VARROA DESTRUCTOR* IN HONEY BEE *APIS MELLIFERA* COLONIES

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ABSTRACT

For present study mustard stem powder and mustard stem ash (*Brassica juncea*) (5g/ colony) were used against *Varroa* in *Apis mellifera* colonies by dusting on the top bar of the frames in replicated hives. Mustard powder and ash treatment were effective in increasing the natural mite fall as significantly lower count (14 and 28.5 mites/hive) was recorded in the residual treatment than in untreated hives which showed higher residual *Varroa* population (105 mites/hive). In mustard powder treatment brood area also increased significantly (cd = 125.0; p= 0.05), whereas in mustard stem ash some increase in pollen area was observed (cd = 5.93; p= 0.05) over control. Other parameters such as bee strength and honey area had no significant change as compared to control. Overall, Mustard powder had more efficacy (77.9%) and percent reduction over control (65%) as compared to mustard stem ash (69.8 % efficacy and 65% reduction over control) against *Varrosis* in *Apis mellifera*.

KEY WORDS
Apis mellifera
Bioinsectcide
Mustrad stem ash
Mustard stem powder, *Varroa*.

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INTRODUCTION

Varroa destructor Anderson and Trueman has direct impact on developing and adult *Apis mellifera* L., resulting in lowered body weights and reduced longevity. It is reported to cause 30-40 per cent loss in *A. mellifera* colonies (Anonymous, 2006) causing structural deformity to upto 3% adult bees (Asha *et al.,* 2013). In the present scenario, 90 per cent apiaries and 50 per cent colonies are affected by this mite in India (Gulati *et al.*, 2009). Environmental factors have a significant impact on the population of *Varroa* in *Apis mellifera* Colonies in North Indian conditions (Kotwal *et al.,* 2013; Poonia *et al.,* 2014).

Several control measures are reported in literature which include use of screen Floor and powdered sugar (Asha *et al.*, 2009), organic acids (formic acid and oxalic acid) (Asha *et al.*, 2014), chemicals (Fluvalinate, Flumethrin, Amitraz, Cymiazole, Coumaphos, Bromoprophylate) and many vegetable oils etc. against *V. destructor* in *A. mellifera* (Mahmood *et al.,* 2012). Synthetic chemicals, although most effective and reliable as they provide immediate relief but cannot be used in organic honey production because of high residue levels in honey (Gulati and Kumari, 2005) and problem of development of resistance in *Varroa* (Colin *et al.,* 1997). Therefore, attention is diverted for other alternatives such as destruction of drone brood, caging of queen, use of botanicals, essential oils, biocontrol agents, resistant bees (Danka *et al.,* 2013), fungal treatment (*Metarhizium anisopliae, Beauveria, Hirsutella, Paecilomyces, Tolypocladium, Lecanicillium)* etc. (Pirali-kheirabadi, 2013). Still, there is lack of efficient control methods currently available against the parasite new approaches are needed for its control (Dietemann *et al.,* 2012). Mustard (*Brassica juncea*) is most commonly used oilseed for food and fodder. Mustard was chosen due to presence of sulphur (each gram of mustard stem powder has 1.44 to 5.22 mg of sulphur), phenols and glucosinolate, which are toxic to soil borne pathogens (Antonious *et al.,* 2009) and may be detrimental to *V. destructor* too. Hence, the present study was designed with the objectives, to know the efficacy of Bioinsectcides (Mustard stem Powder and Mustrad stem Ash) against *V. destructor* in *A. Mellifera,* to know effect of these Bioinsectcides on colony strength (honey bee strenth, brood area) and colony stores (honey area, pollen area).

MATERIALS AND METHODS

Mustard stem powder and ash preparation and treatment

Mustard powder was prepared from fine powder of left over stems of mustard crop (*Brassica juncea*). Mustard ash was prepared by burning the left over stems of the crop (*Brassica juncea*). Both were dusted on the top bar of the frames of the hive at the rate of 5g/hive. Both treated and untreated groups were replicated thrice and were randomly distributed in the apiary. *Brassica juncea* was taken

from CCS Haryana Agricultural University field for experiment

Pre treatment count

Sticky paper was inserted on to the bottom board of experimental colonies. Sticky papers were removed three days later and natural mite fall was quantified (Ostiguy *et al.,* 2000; Dietemann *et al.,* 2013).

Colony Selection

On the basis of pretreatment count, uniform pairing of treated and untreated colonies was done having non significant mite, bee population and brood, honey, pollen area between them. Prior to experimentation, the worker populations were equalized for bees so that each hive contained approximately 5 frames of bees (Dietemann *et al.,* 2013). Brood, honey and pollen area were quantified in square centimeters on all frames using wire grid having squares of 2.5 cm on a side (Harbo and Harris, 2004; Dietemann *et al.,* 2013). The data were compared with *V. destructor* infested colonies where no treatment was given.

Post treatment count

Fresh white sticky paper on the bottom board was placed in each test colony. The number of mites in hive was estimated on sticky paper at each observation period *i.e.* 7, 14 and 21 days after treatment. At each observation period, old sticky paper was replaced with new to avoid the confusion in counting the number of earlier dropped mites over latest mite drop per hive.

Final treatment count

A. mellifera colonies were treated with formic acid (5 ml of 85%) by cotton swab method after 21 days. Mites were collected from the bottom of hives using sticky paper method in both treated and untreated groups.

Per cent efficacy and per cent reduction in mite population over control were calculated by formulae following the method of Dietemann *et al.* (2013) and Eguaras *et al.* (2005), respectively.

RESULTS

Efficacy of dry mustard stem powder

Before the application of dry stem powder of mustard @ 5g/ hive, natural *V. destructor* infestation in the hives was 13.5 mites/ hive. No significant difference was recorded for natural infestation of *Varroa* between the treated and untreated hives. Mustard powder application on top bar led to significant (CD = 3.34; p = 0.05) increase in natural fall of *V. destructor* (63 mites/hive) at the end of three week period as compared to 46.9 mites/hive in untreated hives (Table 1). Week wise, post treatment count was 21, 21 and 21 mites/ hive in first, second and third week, respectively which was more than 13.3, 16.6 and 17 mites/hive in similar weeks in untreated *A. mellifera* colonies. Mustard powder treatment was effective in increasing the natural mite fall as significantly lower count (14 mites/hive) was recorded in the residual treatment (CD = 8.86; p = 0.05) than in untreated hives which showed higher residual *V. destructor* population (105 mites/hive) (Table 1). The per cent efficacy and per cent control over untreated hives was 77.95 and 65.06, respectively in dry stem mustard powder treatment.

During the course of this study, a significant increase (CD = 125; p = 0.05) in brood area (572 to 780 cm^2) was recorded

Table 1: Efficacy of Mustard stem powder against *Varroa destructor* in *Apis mellifera* colonies

Treatment	PreTreatment	Number of mites/hive after treatment on sticky paper					
		7 DAT	14 DAT	21 DAT	Total	Mean after treatment	After final Treatment*
Mustard stem powder @ 5g/hive	13.50	21.00	21.00	21.00	63.00	21.00	14.00
Control	13.00	13.30	16.60	17.00	46.90	15.60	105.00
CD (p = 0.05)	N.S.				3.34		8.86

DAT = Days after treatment; *Formic acid (5 ml of 85%) was applied to record residual mite count

Table 2: Effect of Mustard stem powder on colony strength and stores in *Apis mellifera* colonies

Treatments		Bee strength (frames)	Brood Area (cm^2)	Honey (g)	Pollen Area(cm^2)
Pre treatment	Mustard stem powder @ 5g/hive	4.0	572.0	195.0	117.0
	Control	4.5	560.0	195.0	109.0
	CD (p = 0.05)	N.S.	N.S.	N.S.	N.S.
Post treatment	Mustard stem powder @ 5g/hive	6.5	780.0	280.0	65.5
	Control	4.7	569.5	237.5	65.0
	CD (p = 0.05)	N.S.	125.0	N.S.	N.S.

NS = Non-significant

Table 3: Efficacy of Mustard stem ash against *Varroa destructor* in *Apis mellifera* colonies

Treatment	PreTreatment	Number of mites/hive after treatment on sticky paper					
		7 DAT	14 DAT	21 DAT	Total	Mean after treatment	After final Treatment*
Mustard stem ash @ 5g/ hive	12.00	28.00	20.50	29.50	78.00	26.00	28.50
Control	13.00	13.30	16.60	17.00	46.90	15.63	105.00
CD(p = 0.05)	N.S.				8.61		9.59

DAT = Days after treatment; *Formic acid (5 ml of 85%) was applied to record residual mite count

Table 4: Effect of Mustard stem ash on colony strength and stores in *Apis mellifera* colonies

Treatments		Bee strength (frames)	Brood Area (cm^2)	Honey (g)	Pollen Area(cm^2)
Pre treatment	Mustard ash @ 5g/hive	5.2	644.0	163.7	64.5
	Control	4.5	676.6	145.7	55.0
	CD (p = 0.05)	N.S.	N.S.	N.S.	N.S.
Post treatment	Mustard ash @ 5g/hive	5.2	805.5	228.3	25.0
	Control	4.5	801	223.7	8.0
	CD (p = 0.05)	N.S.	N.S.	N.S.	5.93

NS = Non-significant

after treatment as compared to brood area in untreated *A. mellifera* colonies (560 to 569.5 cm^2) (Table 2). Although, bee strength and honey showed an increase from 4 to 6.5 frames and 195 to 280 g, respectively and pollen area decreased from 117 to 65.5 cm^2 but they did not differ significantly with the corresponding bee strength (4.5 to 4.7 frames), honey (195 to 237.5 g) and pollen area (109 to 65 cm^2) in control.

Efficacy of mustard stem ash

The 5.2 frames bee strength dusted with mustard stem ash @ 5g/hive had an average pretreatment *V. destructor* count of 12 mites/hive which was statistically comparable with 13 mites/ hive in untreated hives (Table 3). The treatment effect was significant (CD = 8.61; p = 0.05) as significantly more number of mites (78 mites/hive) were detected on sticky paper in treated hives as compared to 46.9 mites/hive in untreated hives. Week wise, mite fall on sticky paper was 28, 20.5 and 29.5 mites/hive in first, second and third week of treatment, respectively. In contrast, in untreated *A. mellifera* colonies, natural mite fall was 13.3, 16.6 and 17 mites/hive during the same period. After three weeks, treatment with 5ml of formic acid (85%) to obtain final count resulted in 28.5 mites/ hive on sticky paper. In untreated hives, similar residual treatment showed significantly higher mite fall (105 mites/hive) on sticky paper (CD = 9.59; p = 0.05) (Table 3) which also depicted the effectiveness of the treatment. The per cent efficacy and per cent reduction in *V. destructor* population over untreated hives was 69.84 and 65.06, respectively.

Observations were also recorded on colony strength and stores in *A. mellifera* colonies before and 21 days after the treatment. As colonies were equalized before the treatment, treated and untreated hives showed comparable data on bee strength, brood, pollen area and honey (Table 4). It is evident from data that although brood area and honey increased from 644 to 805.5 cm^2 and 163.7 to 228.3 g/hive in treated hives but no significant difference was observed with untreated hives (801 cm^2 brood and 223.7 g honey). Bee strength also remained at par in both the treated (5.2 frames) and untreated hives (4.5 frames). Pollen area in treated hives decreased from 64.5 to 25 cm^2 and in control 55 to 8 cm^2 and showed significant differences between the treatments (CD = 5.93; p = 0.05).

DISCUSSION

Chemical resistance, contamination of bee products and variable efficacies of current *Varroa* treatments create a need for alternative treatment methods (Dietemann *et al.*, 2012). Due to highly resistant nature of *Varroa* against chemical treatments, repeated applications of same chemical cannot be given (Rosenkranz *et al.*, 2010). Thus, mustard stem powder/ash application was chosen to test against Varrosis as it does not harm the bees but does interfere with the mites' ability to maintain its hold on the bee. Bending and Lincoln (1999) have observed that *Brassica juncea* releases highly toxic isothiocyanates, and large quantities of mildly toxic non-glucosinolate derived volatile S-containing compounds during decomposition, which lead to 'biofumigant' effect resulting in suppression of a variety of soil-borne plant pathogens and pests. Phenols and glucosinolatepresent in *Brassica juncea* were found toxic to soil borne pathogens by Antonious *et al.* (2009). Hence, all these compounds may be detrimental to *V. destructor* also.

In the present study, mustard stem powder/ash application on top bar led to significant (CD = 4.71, 8.61; p = 0.05 for dry mustard stem powder and ash, respectively) increase in natural fall of *V. destructor* at the end of three week period. Sulphur powder is highly effective against *Varroa* alone and in combination with other chemicals (Sakai and Takeuchi, 1980; Kotwal and Abrol, 2013), and *Brassica juncea* has high percentage of sulphur which may also have negative effect on *Varroa*. But definitely some constituents of stem powder which are effective against mites are lost in ash as per cent efficacy and per cent control over untreated hives were found to be more in dry stem mustard powder (77.95 and 65.06, respectively) than mustard ash (69.84 and 65.06, respectively).

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