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Bio-efficacy of some botanical leaf extracts against mustard aphid, *Lipaphis erysimi* (Kaltenbach) and their influence on the yield and yield attributes of Indian mustard (*Brassica juncea*)

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Abstract
The current study was undertaken to assess the bio-efficacy of leaf extracts of Neem (*Azadirachta indica* A. Juss), Tobacco (*Nicotiana tabacum* L.), African marigold (*Tagetes erecta* L.), a mixture of the three extracts, imidacloprid, and a control (water spray) against mustard aphid during two consecutive Rabi seasons of 2022-23 and 2023-24. The percentage reduction over control (PROC) values were calculated for each botanical extract to ascertain their effectiveness at 1, 3, 7 and 10 days after treatment. The pooled efficacy at 10 days after treatment revealed that Imidacloprid was the most potent, recording 100.0% reduction, followed by neem leaf extracts (82.79%), tobacco leaf extracts (78.76%), and the mixture treatment (77.41%), with marigold leaf extract displaying the lowest efficacy at 69.01%. A parallel trend was observed in seed yield, affirming the efficacy of these treatments in mitigating aphid-induced losses in mustard crops. The botanical extracts showed significant efficacy potentials and so can be used as substitutes for imidacloprid insecticide to manage the aphid in an eco-friendlier manner.

Keywords: Mustard, *Lipahis erysimi*, imidacloprid, botanicals

Introduction

India's rapeseed and mustard productivity which currently stands at 1397 kg/ha, falls short of the global average of 1849 kg/ha (Kaur and Grover, 2020) [11]. This low productivity has to a higher extent been attributed to the fact that the crop suffers high yield losses accruing from various biotic and abiotic stresses (Patel *et al.*, 2017) [15]. India has reported over 43 species of insect pests in rapeseed-mustard crops to date. Among them, major pests include the aphid (*Lipaphis erysimi*), painted bug (*Bagrada hilaris*), leaf miner (*Phytomyza horticola*) and sawfly (*Athalia lugens proxima*). The mustard aphid alone has been documented to result in yield losses of up to 96 percent and a reduction in oil content by 5-6 percent. (Sahoo, 2012; Lal *et al.*, 2018) [12, 18]. The aphid attacks on mustard leaves produce yellowing, curling, and drying symptoms which results in the production of small and shriveled seeds in mustard siliquae. It slows down the rate of photosynthesis and secretes honeydew, leading to the development of sooty mold (Patel *et al.*, 2017) [15]. The single-stranded RNA luteoviruses transmission has been associated with the phloem sap-sucking habits of mustard aphids (Banerjee *et al.* 2004) [4]. Several chemical insecticides have been found useful across the country against mustard aphids. Though highly effective, these insecticides not only harm natural predators of aphids like coccinellids, *Diaeretiella rapae*, *Chrysoperla zastrowi arabica* and syrphid flies (Singh *et al.*, 2007) [19], but they also contribute to environmental pollution, health risks, pollinator toxicity, and residues in cake and oil (Singh and Sharma, 2002) [19]. Pesticides derived from plants are largely comprised of secondary metabolites which plants produce in a defense response to herbivory. A large proportion of these secondary metabolites are members of lactones, fatty acids, essential oils, alkaloids, steroids, isoflavonoids, pterocarpans, lignans, alkanes, alkenes, alkynes, and simple aromatics (El-Wakeil, 2013) [9]. In certain insect species, these chemical plant extracts exhibit lethal, anti-feedant, and growth-disturbing properties through inhibiting molting (Al-Fifi, 2009) [1]. Recent years have seen a rise in the use of botanicals for pest control since they are eco-friendly, cheap, more readily available, and relatively less poisonous to natural enemies. The current study was conducted to examine the potency of selected botanicals in comparison with a conventional insecticide against the aphid, *L. erysimi* on Indian mustard.

Materials and methods

The field experiment was conducted at the Research Farm of the Faculty of Agriculture, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala, Haryana during the Rabi seasons of 2022-2023 and 2023-2024. Situated at 7703'0"E longitude and 30017'0"N latitude, the site is characterized by a semi-arid climate of that includes a warm, dry summer (up to 48^0C) and very cold winter (-1^0C) and experiences a total annual rainfall of 1193.8mm out of which 70-80% occurs between July and September, with the remaining 20-30% from December to February. Seeds of a susceptible mustard variety, RH 0119 were sown during the second week of November in a randomized block design (RBD) with three replications at a spacing of 45 x 10 cm in plots of 2.5 x 4 $m^{2.}$ Good agronomy practices were adopted to raise a robust mustard crop. Detailed treatments are presented in Table 1.

Preparation of botanical leaf extracts

Fresh leaves of neem (*Azadirachta indica* A. Juss) and marigold (*Tagetes erecta* L) were collected from the orchard and horticulture garden of MM (DU), Mullana, respectively while leaves of tobacco **(***Nicotiana tabacum* L) were procured from farmers' fields at Mullana village. These were thoroughly cleaned and evenly spread in the Entomology laboratory for three days to dry under shade before being transferred to an open place to completely dry under sun heat for other four days. Dried leaves were ground into powder with the help of a mechanical grinder. The powder was then passed through a 30 mesh-sized sieve to get rid of large ungrounded pieces of the materials and then loaded into a sox let apparatus. Running in batches, 60 g of each powder was loaded into respective thimbles, onto which 70 ml of ethanol was added through to the flat-bottomed flask. This was left to run concurrently for 48 hours with temperature maintained at 60 ^{0}C. The procedure was repeated until the required quantity of concentrated crude extract (33 g) was obtained. Details of the total amount of powder and ethanol used, together amount of concentrated crude extract recovered for each botanical and the percentage purity of each active ingredient are presented in Table 2.

Foliar sprays @ 5% crude extract of each botanical were applied against mustard aphids as soon as average populations reached 25-50 aphids/10 cm terminal shoots in the field. For mixture treatment (TLE+NLE+MLE)), dilution was made to 3%, by firstly mixing the three crude extracts in a ratio of 1:1:1 to make up to 3g and then dissolving it in 100 ml of water. Forty-four grams of each botanical treatment was required to spray the triplicated set of plots. The insecticide treatment was made by diluting 0.5 ml of imidacloprid in one litre of water (0.5 ml/l) while the control treatment was a simple water spray. These dilutions were applied with the help of a 10-litre Knapsack sprayer, which was always thoroughly cleaned after every treatment to avoid cross-contamination. By counting number of dead aphids (adults and nymphs) on 10 randomly tagged plants per plot, the percentage reduction of population over control (PROC) was calculated at 1, 3, 7 and 10 days after treatment by Henderson and Tilton's (1955) formula to determine the efficacy of these treatments. *Viz.*

$$PROC = [1- \{(Ta \times Cb) / (Tb \times Ca)\}] \times 100$$

Where, Tb = Population in treatment before spray, Ta = Population in treatment after spray, Cb = Population in control before spray and Ca = Population in control after spray. As per Gomez and Gomez (1984), the PROC data was analyzed using a one-way ANOVA and the OPSTAT software. Subsequently, Duncan's Multiple Range Test (DMRT) at a significance level of 5% was employed to compare means.

Results

The results for each year of study and the pooled bio-efficacy from the two years' study have been presented separately (Tables 3-5). The overall efficacy (pooled bio-efficacy at the final time of evaluation) *viz.* 10 days after treatment (DAT) revealed that all treatments reduced pest populations significantly over control. The aphid populations remained at par under TLE (22.46) and NLE (21.63) but were significantly different among the rest of the treatments. The highest aphid population remained in control (112.60) while none in imidacloprid-treated plots. All treatments registered their highest potency, with a maximum bio-efficacy of 100% recorded under imidacloprid-treated plots while the least remained under control (0.00%). The efficacy for mixture treatment (77.41%) was at par with TLE (78.76%) but not NLE (82.79%) which was significantly highest among the botanicals. The least efficacy among the botanicals was recorded under MLE (69.01%). The efficacy was in the descending order as imidacloprid > NLE > TLE > Mixture (TLE+NLE+MLE) > MLE > control, as had been observed in the respective years of evaluation.

From Table 6, the highest number of siliquae per plant was recorded under imidacloprid treatment (464.00) which was at par with NLE (440.33) which recorded the highest siliquae per plant among the botanicals. The significantly least number of siliquae per plant i.e. 396.00 and 420.66 was observed under MLE and Mixture treatment (statistically at par). Imidacloprid and NLE-treated plots had significantly more seeds per siliquae (13.67 and 13.22) followed by TLE (11.78) while other treatments did not differ significantly in this regard. The highest test weight was recorded under imidacloprid (4.31g) and NLE (4.08g) treatments which was statistically at par with TLE and mixture treatments. However, the least test weight was recorded in control.

All treatments significantly reduced the number of malformed siliquae over control. The lowest percentage of malformed siliquae was recorded in imidacloprid (9.61%) and NLE (11.83%) which were statistically at par. This was followed by TLE, mixture, and MLE, being at par with each other. For seed yield, all treatments were significantly different from the control. The highest seed yield was recorded under imidacloprid treatment (28.53 q/ha) and the least under control (19.11 q/ha). Among the botanicals, the highest yield was obtained under NLE (24.50 q/ha) which was at par with that under TLE (23.79 q/ha) while the least was obtained under mixture treatment (22.38 q/ha) and MLE (21.35 q/ha).

Discussion

The high bio-efficacy (100%) reported under imidacloprid treatment is similar to the findings of Patel *et al.* (2017) [15]

and Patel and Hasan (2020) [14] who also reported the highest aphid mortality (100%) after 7 days. Dotasara *et al.* (2017) [8] reported up to 87.55% aphid mortality under imidacloprid which was still the highest in this treatment. In all these studies, the highest seed yield was reported under the same treatment, confirming the superiority of imidacloprid in the management of mustard aphids. the current bio-efficacy results conform to those of Bhatta *et al.* (2019) [5] who reported 88.6% aphid mortality for neem leaf extract treatment, applied at 5% concentration but differ from those of Chattree *et al.* (2016) [6] who recorded maximum aphid mortality of 92.85% in first larval instar of mustard aphid when sprayed with 20% neem leaf extract. The obvious explanation for the difference could be owing to the higher concentration of the extract taken by the later author. However, Aziz *et al.* (2014) [3] reported up to 100% mortality for plants sprayed with 2.5% neem oil, followed by 86.13% mortality for 10% neem seed cake extract, indicating the superiority of these neem formulations over neem leaf extracts in managing mustard aphids. The present investigation for tobacco leaf extract bio-efficacy (78.28%) is in agreement with those of Bhatta *et al.* (2019) [5] and Essani *et al.* (2020) [10] who found 71.38% and 88.16% aphid mortalities respectively. However, Sable and Kushwaha (2014) [17] observed only up to 63.0% aphid mortality under the same treatment. The lowest efficacy under marigold leaf extract (68.6%) aligns with the results of Dhaked *et al.* (2016) [7] who reported 67.95% mustard aphid mortality with treatment of 5% marigold leaf extract. Pal *et al.* (2020) [13] registered the maximum aphid reductions under complete mixture treatment comprising Neem (*Azadiractin indica*), Hattibar (*Agave americana*), Khirro (*Sapium insigne*), Bakaino (*Melia azedirach*), Tobacco (*Nicotiana tabaccum*) and Bojho (*Acorus calamus*) that were at par with the cypermethrin 10% EC. They further noted that omitting neem from the extract mixture produced an effect similar to that of control, signifying the need for neem in all plant extract mixture ratios for the effective management of mustard aphids. In the current study, the mixture treatment recorded an efficacy better than marigold leaf extract but lower than tobacco leaf extract, neem leaf extract, and imidacloprid.

Table 1: Plant extracts evaluated against *Lipaphis erysimi*

Treatment number	Treatment	Dosage
T1	Tobacco leaf extract (TLE)	5%
T2	Neem leaf extract (NLE)	5%
T3	Marigold leaf extract (MLE)	5%
T4	Mixture treatment (TLE+NLE+MLE)	3%
T5	Imidacloprid	0.5 ml/l
T6	Control	---

Table 2: Amount of crude extracts recovered from different botanicals

Botanical	Total amount of botanical used (g)	Total amount of ethanol used (ml)	Total amount of crude extract recovered (g)
Neem	421	491	44
Tobacco	215	251	44
Marigold	561	655	44

Table 3: Bio-efficacy of different botanical leaf extracts for mustard aphid control during 2022-23 Rabi season

Treatment	1DBT	1DAT		3DAT		7DAT		10DAT	
	AVG	AVG	PROC	AVG	PROC	AVG	PROC	AVG	PROC
Tobacco Leaf extract (TLE) @5%	109.21d (10.45)	56.33d (7.50)	48.41b (6.99)	44.22d (6.65)	60.69b (7.82)	35.63d (5.97)	68.25b (8.29)	26.53d (5.19)	76.78bc (8.79)
Neem leaf extract (NLE) @5%	114.66d (10.71)	57.43d (7.58)	49.19b (7.04)	38.22e (6.18)	66.80b (8.19)	32.95d (5.74)	70.98b (8.45)	23.13e (5.32)	81.15b (9.03)
Marigold leaf extract (MLE) @5%	143.78b (11.99)	98.76b (9.93)	31.50d (5.65)	74.11b (8.61)	49.70c (7.08)	62.23b (7.89)	57.51c (7.61)	53.36b (7.34)	65.06d (8.09)
Mixture (TLE +NLE +MLE) @3%	124.11b (11.14)	70.80c (8.41)	42.38c (6.54)	58.44c (7.64)	53.80c (7.36)	42.19c (6.49)	65.52bc (8.12)	33.63c (5.84)	74.51c (8.66)
Imidacloprid @0.02%	158.44a (12.59)	33.33e (5.77)	78.94a (8.91)	12.88f (3.56)	92.18a (9.63)	6.66e (2.57)	95.88a (9.81)	0.00f (0.00)	100.00a (10.02)
Control	114.65d (10.71)	114.66a (10.71)	0.00e (0.00)	118.10a (10.87)	0.00d (0.00)	118.10a (10.87)	0.00d (0.00)	122.90a (11.11)	0.00e (0.00)

Means that have been followed by the same letters did not differ significantly at $p<0.05$ by DMRT.

Table 4: Bio-efficacy of different botanical leaf extracts for mustard aphid control during 2023-24 Rabi season

Treatment	1DBT	1DAT		3DAT		7DAT		10DAT	
	AVG	AVG	PROC	AVG	PROC	AVG	PROC	AVG	PROC
Tobacco Leaf extract (TLE) @5%	87.23a (9.34)	53.10c (7.29)	39.02c (6.27)	42.47bc (6.51)	53.38c (7.33)	28.67c (5.34)	68.65c (8.31)	18.43cd (4.34)	80.72bc (8.96)
Neem leaf extract (NLE) @5%	92.34a (9.61)	48.57c (6.97)	47.36b (6.92)	35.10c (5.92)	63.73b (8.01)	24.00c (4.89)	75.20b (8.70)	15.43d (3.99)	84.85b (9.19)
Marigold leaf extract (MLE) @5%	96.62a (9.83)	71.47b (8.45)	25.99e (5.15)	51.23b (7.16)	49.78c (7.08)	41.20b (6.42)	59.24e (7.73)	30.86b (5.59)	71.00d (8.37)
Mixture (TLE +NLE +MLE)@3%	100.37a (10.02)	67.00b (8.18)	33.24d (5.78)	51.00b (7.14)	48.63c (7.00)	37.10b (6.09)	64.70d (8.08)	23.73bc (4.92)	78.62c (8.83)

Imidacloprid @0.02%	87.44a (9.35)	23.44d (4.83)	73.06a (8.57)	15.37d (3.92)	83.17a (9.15)	2.57d (1.60)	97.19a (9.88)	0.00e (0.00)	100.00a (10.00)
Control	92.38a (9.59)	92.38a (9.59)	0.00f (0.00)	96.93a (9.82)	0.00d (0.00)	96.93a (14.38)	0.00f (0.00)	102.33a (14.58)	0.00e (0.00)

Means that have been followed by the same letters did not differ significantly at $p<0.05$ by DMRT.

Table 5: Pooled bio-efficacy of different botanicals against mustard aphid

Treatment	1DBT	1DAT		3DAT		7DAT		10DAT	
	AVG	AVG	PROC	AVG	PROC	AVG	PROC	AVG	PR0C
Tobacco Leaf extract (TLE) @5%	98.23c (9.91)	53.10c (7.29)	44.45bc (6.55)	43.37d (6.58)	57.04bc (7.58)	32.13d (5.67)	68.45c (8.30)	22.46d (4.79)	78.76bc (8.90)
Neem leaf extract (NLE)@5%	103.53c (10.18)	48.57c (6.97)	48.28b (6.98)	36.67e (6.05)	63.73b (8.01)	28.50d (5.34)	73.10b (8.58)	21.63d (4.70)	82.79b (9.13)
Marigold leaf extract (MLE) @5%	120.20a (10.96)	71.47b (8.45)	28.75d (5.40)	62.70b (7.92)	49.99c (7.10)	51.73b (7.19)	58.38d (7.67)	42.16b (6.53)	69.01d (8.33)
Mixture (TLE +NLE +MLE) @3%	112.26b (10.59)	67.00b (8.19)	37.96c (6.19)	54.73c (7.39)	53.58c (7.34)	39.67c (6.29)	65.12c (8.09)	28.73c (5.41)	77.41c (8.83)
Imidacloprid @0.02%	122.93a (11.09)	23.43d (4.83)	74.14a (8.63)	14.10f (3.75)	87.68a (9.39)	4.60e (2.14)	96.54a (9.85)	0.00e (0.00)	100.00a (10.00)
Control	103.53c (10.17)	92.43a (9.59)	0.00e (0.00)	107.53a (10.36)	0.00d (0.00)	107.53a (10.36)	0.00e (0.00)	112.60a (10.63)	0.00e (0.00)

Means that have been followed by the same letters did not differ significantly at $p<0.05$ by DMRT.

Table 6: Pooled effect of evaluated plant extracts on seed yield and yield attributes

Treatment	Seed yield / yield attribute evaluated				
	No. of siliquae per plant	No. of seeds per siliquae	%.malformed siliquae per plant	1000-seed weight (g)	Seed yield (q/ha)
Tobacco Leaf extract (TLE) @5%	434.33b (20.84)	11.78b (3.43)	12.83b (3.58)	4.00abc (2.00)	23.79bc (4.88)
Neem leaf extract (NLE) @5%	440.33ab (20.98)	13.22a (3.63)	11.83bc (3.43)	4.08ab (2.02)	24.50b (4.95)
Marigold leaf extract (MLE)@ 5%	396.00c (19.90)	10.68b (3.26)	13.46b (3.67)	3.62bc (1.89)	21.35d (4.62)
Mixture (TLE+NLE+MLE) @3%	420.66bc (20.51)	10.89b (3.29)	13.15b (3.61)	3.84abc (1.96)	22.38cd (4.73)
Imidacloprid @0.02%	464.00a (21.53)	13.67a (3.69)	9.61c (3.09)	4.31a (2.07)	28.53a (5.34)
Control	333.33d (18.26)	10.89b (3.29)	25.28a (5.02)	3.55c (1.88)	19.11e (4.37)

Means that have been followed by the same letters did not differ significantly at $p<0.05$ by DMRT.

Conclusion

In summary, the mixture treatment (3%) and sole treatments of tobacco leaf extract (5%) and neem leaf extract (5%) can be used as an alternative to the insecticide, imidacloprid (0.5%) to minimize the negative effects of pesticides to the environment and food chains. To increase the potential of marigold leaf extract as a botanical insecticide against aphids, it can be used in combinations with either neem or tobacco or all mixtures at 5% concentration. Another trial, exhausting all possible combinations of botanicals at similar and/or different concentrations could reveal the actual joint action mechanism that underlies them.

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