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Incorporation of biorational insecticides with neonicotinoids to combat resurgence of *Tetranychus urticae* (Prostigmata: Tetranychidae) on rose

Garima Gupta

Abstract

Rose plants were treated with 2 neonicotinoids (imidacloprid and acetamiprid), 3 biorationals (spinosad, emamectin benzoate, and *Beauveria bassiana* [Bals.-Criv.] Vuill. [Hypocreales: Cordycipitaceae]), and combinations of neonicotinoids and biorationals to control two-spotted spider mites (*Tetranychus urticae* Koch; Prostigmata: Tetranychidae) on rose. Toxicity bioassays revealed that imidacloprid drench, imidacloprid foliar applications, and acetamiprid treatments significantly increased spider mite numbers, whereas treatments with spinosad or with emamectin either alone or in combinations with imidacloprid and acetamiprid resulted in significantly fewer spider mites compared with the untreated control. *Beauveria bassiana* was least effective in controlling the spider mite population. The fecundity experiment indicated that the imidacloprid drench and foliar treatments significantly increased the number of mite eggs produced during observation days up to 9 d after treatment (DAT) for the drench treatment and up to 7 DAT for the foliar treatments. Acetamiprid did not induce any significant changes in spider mite egg production. Spinosad, emamectin, and *B. bassiana* alone or in combinations induced significant reductions in egg numbers compared with the control at almost all observation days. Both preference and non-preference tests indicated that the drench and the foliar applications with imidacloprid resulted in significantly more spider mites on treated leaf discs at 5 and 7 DAT than on untreated leaves. In conclusion, spinosad and emamectin in combinations with the neonicotinoids can be incorporated into the integrated pest management of the two-spotted spider mite on roses.

Key Words: emamectin; imidacloprid; spinosad; *Beauveria bassiana*

Resumen

Plantas de rosas fueron tratadas con 2 neonicotinoides (imidacloprid y acetamiprid), 3 biorracionales (spinosad, benzoato de emamectina y *Beauveria bassiana* [Bals.-Criv.] Vuill. [Hypocreales: Cordycipitaceae]), y combinaciones de neonicotinoides y biorracionales para el control de la arañita roja de dos manchas (*Tetranychus urticae* Koch; Prostigmata: Tetranychidae). Los bioensayos de toxicidad revelaron que el tratamiento de imidacloprid remojado y de imidacloprid foliar y los tratamientos de acetamiprid resultó en un aumento significativo en el número de arañitas rojas, sin embargo, spinosad y emamectina, solos o en combinaciones con imidacloprid y acetamiprid resultó en un número significativamente menor de arañitas rojas que en el control no tratado. *Beauveria bassiana* fue el menos eficaz en el control de la población de arañitas rojas. El experimento de la fecundidad indicó que los tratamientos de imidacloprid remojado o foliar aumentaron significativamente el número de huevos de ácaros durante los días de observación hasta 9 DAT (dias después de tratamiento) para el remojado y hasta 7DAT para los tratamientos foliares. Acetamiprid no indujo cambios significativos en la producción huevos de acaros. Spinosad, emamectina y B. *bassiana* solos o en combinaciones, indujo reducciones significativas en el número de huevos en comparación con el control en casi todos los días de observación. Ambas pruebas de preferencia y no preferencia, indicaron que el remojado con imidacloprid y las aplicaciones foliares resultó en significativamente mas ácaros en discos de hojas tratadas en 5 y 7 DAT que en las hojas no tratadas. En conclusión, spinosad y emamectina en combinaciones con los neonicotinoides se pueden incorporar en el manejo integrado de plagas de la arañita de dos manchas en las plantas de rosas.

Palabras Clave: emamectin; imidacloprid; spinosad; *Beauveria bassiana*

The two-spotted spider mite, *Tetranychus urticae* Koch (Prostigmata: Tetranychidae), is an extremely polyphagous pest. It attacks almost 200 plant species and causes serious damage to agricultural crops, particularly to herbaceous annuals, such as bean, tree fruits, roses, and other ornamental plants (Kennedy & Storer 2000; Lee et al. 2003). On ornamentals, spider mites are primarily an aesthetic concern, but they can kill plants if populations become very dense. *Tetranychus* species are distributed throughout Asia and North America (Bolland et al. 1998; Navajas et al. 2001). Their high reproductive rate and short life cycle make them serious pests in the field and protected agriculture (Ullah et al. 2011). Many acaricides and insecticides have been recommended for their control, but the matter of concern here is that spider mites have the ability to develop resistance to various agrochemicals after only a few applications (Goka 1998; Devine et al. 2001).

Neonicotinoid insecticides (imidacloprid, acetamiprid, etc.) have lower mammalian toxicity than other new generation insecticides, and this advantage has resulted in broad registrations for their use (Lexmond et al. 2015). Neonicotinoids are used worldwide in seed and soil treatments, and they are formulated for foliar applications to control sucking insects, including aphids, thrips, whiteflies, and fungus gnats (Meister 2000). The systemic properties of imidacloprid allow it to become evenly distributed in the young growing plant (Ishaaya & Degheele 1998). Some studies have suggested that imidacloprid applications may increase mite infestations. Evidence for this was reported concerning hops (James & Price 2002), hemlock (Raupp et al. 2004), marigolds (Sclar et al. 1998; Cranshaw & Sclar 2006), and roses (Gupta & Krischik 2007), on which when treated with imidacloprid mite populations increased many fold.

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Biorational and microbial pesticides have been gaining attention globally as important tools for environmentally benign and safe integrated pest management (IPM; Copping & Menn 2000). Biorational or "reduced risk" insecticides are synthetic or natural compounds that effectively control insect pests, and they are especially valuable because their toxicity to non-target animals and humans is usually low (Hara 2000; Shi 2000). Hence, these compounds are considered important components of IPM programs for controlling mites and other agricultural pests. The primary obstacle to using microbial and biologically derived biopesticides is that they work comparatively slowly and also may decompose rapidly in sunlight (Feely et al. 1992; Jansson & Dybas 1998). The combination of a biorational insecticide with a systemic neonicotinoid may be promising for the rapid and prolonged control of mite populations. Neves et al. (2001) examined the compatibility of various entomopathogenic fungi (*Beauveria bassiana* [Bals.-Criv.] Vuill. [Hypocreales: Cordycipitaceae], *Metarhizium anisopliae* [Metschn.] Sorokin [Hypocreales: Clavicipitatceae], and *Paecilomyces* sp. [Eurotiales: Trichocomaceae]) under laboratory conditions with various neonicotinoids including acetamiprid, imidacloprid, and thiamethoxam.

Some commercially available biopesticides include spinosad, several avermectins, and the fungus *B. bassiana*. Spinosad is produced by fermentation of the bacterium *Saccharopolyspora spinosa* Mertz and Yao (Actinomycetales: Pseudonocardiaceae) and is used against fire ants, lepidopteran larvae, and leaf miners (Bret et al. 1997). The avermectins are streptomycete-derived macrocyclic lactones that have high potencies against insect pests in several orders, phytophagous mites, and the plant-parasitic nematode *Meloidogyne incognita* (Tylenchida: Meloidogynidae) (Ishaaya & Horowitz 1998). *Beauveria bassiana* is a fungus that attacks and kills a variety of immature and adult insects (Ownley et al. 2004).

The purpose of this research was to develop a bio-intensive pest management system to reduce two-spotted spider mite resurgence. The envisioned IPM system should reduce the dosage of neonicotinoids and incorporate the use of microbial pesticides, and the system should be safe to non-target organisms and to the environment of ornamental crops. Two specific objectives were to (1) determine the efficacy of various insecticides alone or in combination against spider mites by observing if spider mite populations in the various treatments increased or decreased, and (2) observe changes in fecundity patterns of the treated spider mites in comparison with untreated ones.

Materials and Methods

BIOASSAY EXPERIMENTS

Eighty plants of a tea hybrid red colored rose (*Rosa* sp.; Rosales: Rosaceae) were obtained on 2 Mar 2011 from a nursery (Ram Nursery, Chandigarh, India) and grown in 12 L pots with standard cultural practices in open space outside the Zoology Department, Panjab University, Chandigarh, India. During the summer, a two-spotted spider mite, *T. urticae*, infestation was observed. The mites were collected and reared in plastic containers (47 × 25 × 17 cm) on fresh rose twigs that were changed every other day. These plastic boxes were kept in an incubator at temperature of 28 to 30 °C and 20% RH. For the bioassays, I used available and registered formulations of 3 biorationals (spinosad, emamectin benzoate, and *B. bassiana*) and 2 neonicotinoids (imidacloprid and acetamiprid), each alone and in combinations of 1 neonicotinoid and 1 biorational. The names, formulations, and rates of these materials and combinations of materials are given in Table 1. Thus, 13 treatments were evaluated in the experiment. Drench and foliar spray treatments (dose rates given in Table 1) with these different insecticides were done on 15 May 2011. Laboratory bioassays by slightly modified

Table 1. Insecticides and combinations of insecticides and biorationals tested for the control of *Tetranychus urticae* on tea hybrid roses.

Insecticide common name	Trade name	Manufacturer	Application rate
Imidacloprid	Confidor 17.8% SL	Bayer Crop Science, Mumbai, India	1 mL/4.5 L water, drench treatment per potted plant
Imidacloprid	Confidor 17.8% SL	Bayer Crop Science, Mumbai, India	0.4 mL/2 L water, spray treatment
Acetamiprid	Dhanpreet 20% SP	Dhanuka Agritech Limited, Gurgaon, India	0.2 g/2 L water, spray treatment
Spinosad	Tracer 45% SC	Dow Agro Sciences, Mumbai, India	0.7 mL/2 L water, spray treatment
Emamectin benzoate	Proclaim 5G	Syngenta India Limited, Mumbai, India	0.2 g/2 L water, spray treatment
Beauveria bassiana	Biosoft	Agriland Biotech Limited, Gujrat, India	8 g/2 L water, spray treatment
Imidacloprid + spinosad	Confidor 17.8% SL + Tracer 45% SC	As mentioned above	0.2 mL Confidor + 0.7 mL Tracer per 2 L water, spray treatment
Imidacloprid + emamectin benzoate	Confidor 17.8% SL + Proclaim 5G	As mentioned above	0.2 mL Confidor + 0.2 g Proclaim per 2 L water, spray treatment
Imidacloprid + *Beauveria bassiana*	Confidor 17.8% SL + Biosoft	As mentioned above	0.2 mL Confidor + 8 g Biosoft per 2 L water, spray treatment
Acetamiprid + spinosad	Dhanpreet 20% SP + Tracer 45% SC	As mentioned above	0.1 g Dhanpreet + 0.7 mL Tracer per 2 L water, spray treatment
Acetamiprid + emamectin benzoate	Dhanpreet 20% SP + Proclaim 5G	As mentioned above	0.1 g Dhanpreet + 0.2 g Proclaim per 2 L water, spray treatment
Acetamiprid + *Beauveria bassiana*	Dhanpreet 20% SP + Biosoft	As mentioned above	0.1 g Dhanpreet + 8 g Biosoft per 2 L water, spray treatment

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procedures of James & Price (2002) were conducted to evaluate the effects of insecticide-treated leaves on spider mite fecundity.

Considering the 2 specific objectives of determining the efficacy of the formulated materials in suppressing populations and the effects of the materials on fecundity, different experiments were planned. The 1st objective of this study was to determine the efficacy of various insecticides alone or in combination against spider mites by observing if spider mite populations increased or decreased. The 2nd objective of this study was to observe changes in fecundity patterns of the treated spider mites in comparison with untreated ones. Therefore, different experiments were planned and conducted.

In the 1st experiment, the main objective was to determine whether treated mite populations decreased or increased. Rose plants were sprayed with the various insecticides and their combinations, and after 24 h, their leaves were obtained for use in bioassays. The second oldest compound leaf was taken randomly and placed in a Petri dish with a watered cotton plug at the twig tip. Six plants for each treatment and 2 leaflets from each plant were taken and each kept in a Petri dish, and in each Petri dish 4 mature female mites were released. Old leaves were replaced with fresh leaves, and observations on mite numbers were taken every alternate day. For each Petri dish, old leaves were kept under observation because they may contain eggs. Newly hatched mites from such eggs were released with the old mites of the same sample.

For the study on fecundity, the experimental setup was the same as above. Leaves were changed, and numbers of eggs were counted and recorded every alternate day. After each observation on egg numbers, the old leaves and eggs were discarded, and only larvae and adult mites were placed on the new leaf.

Each experiment was replicated thrice. Replicates were combined, data were tested for homogeneity using Welch's tests and analyzed using ANOVA, and means were compared using Tukey's HSD test (JMP SAS Institute 2011).

PREFERENCE AND NON-PREFERENCE TESTS

From the bioassay data, it was observed that in some treatments with imidacloprid and acetamiprid, the number of mites increased significantly at particular days after treatment (DAT). Hence, choice and no-choice experiments were planned to check whether mites showed any preference or non-preference for leaves treated with a particular insecticide in comparison with untreated leaves at different times after insecticide application. For this experiment, 24 plants of hybrid red colored rose (*Rosa* sp.) were obtained on 17 Dec 2014 from a nursery (Ram Nursery, Chandigarh, India) and grown in pots. On 16 Mar 2015, imidacloprid (drench and foliar) and acetamiprid treatments were applied on 6 plants for each insecticide at their labeled doses as mentioned in Table 1. Untreated or treated rose leaves were brought to the laboratory at 1, 3, 5, 7, 9, and 11 DAT. Rose leaves were cut into square shape discs (2.5 × 2.5 cm), and 4 leaves were kept in a Petri dish (140 × 20 mm) lined with a moistened filter paper (Whatman filter paper 125 mm). Insecticide-treated and untreated leaf discs were arranged alternately around the circumference of the Petri dish. These were placed in the Petri dish at the diagonal position so that 2 choices (treated vs. untreated) were given to test mites. From each plant, 3 leaves were taken and kept in separate Petri dishes. Observations were recorded 24 h after the release of spider mites. The experiment was repeated with a different population of spider mites. Replicates were combined, and data were analyzed using χ^2 goodness of fit analysis (PROC FREQ) to determine if the choice responses deviated significantly from random choice (1:1, 50%) (JMP SAS Institute 2011).

Results

Toxicity bioassays (Table 2) revealed that imidacloprid drench treatments showed significant increases in mite numbers at 7, 9, and 11 DAT compared with the untreated control. Similarly in the case of imidacloprid foliar applications, significant increases in mite numbers were noticed at 9 and 11 DAT. Also, the acetamiprid treatment showed significant increases in mite numbers at 9 and 11 DAT.

Spinosad and emamectin alone and in combinations with imidacloprid and acetamiprid significantly reduced mite numbers at all DAT (Table 2). However, *B. bassiana* showed significant moderate toxicity only at 5 and 11 DAT. *Beauveria bassiana* in combination with acetamiprid significantly reduced mite numbers only at 1, 3, and 5 DAT. Also, *B. bassiana* in combination with imidacloprid significantly reduced mite numbers only at 5 DAT. The data (Table 2) revealed that imidacloprid drench, imidacloprid foliar spray, and acetamiprid treatments significantly increased

Table 2. Mean (± SE) number of mites in the various treatments at the various days after treatment (DAT). Observations were made on alternate days. After each observation, the old leaves and all eggs were discarded, and only mites were placed on the new leaf. Data were analyzed by ANOVA (JMP SAS Institute 2011).

Treatment	1 DAT	3 DAT	5 DAT	7 DAT	9 DAT	11 DAT
Control	4.0 ± 0.0a	3.9 ± 0.1a	3.9 ± 0.1a	7.6 ± 0.5b	8.8 ± 0.3c	10.3 ± 0.3d
Imidacloprid drench	4.0 ± 0.0a	3.9 ± 0.1a	4.0 ± 0.2a	9.5 ± 0.7a	11.8 ± 0.8a	15.3 ± 0.5a
Imidacloprid foliar	4.0 ± 0.0a	3.8 ± 0.1a	3.7 ± 0.1ab	7.5 ± 0.7ab	11.1 ± 0.8ab	13.0 ± 0.9ab
Acetamiprid	4.0 ± 0.0a	3.7 ± 0.2a	3.6 ± 0.2ab	7.4 ± 0.4ab	9.9 ± 0.4b	11.2 ± 0.4c
Spinosad	3.1 ± 0.2b	1.9 ± 0.2ef	0.7 ± 0.2e	0.7 ± 0.2e	1.1 ± 0.5f	1.3 ± 0.7fg
Beauveria bassiana	3.7 ± 0.2a	3.2 ± 0.2ab	2.8 ± 0.2bc	6.0 ± 0.8bc	8.1 ± 1.0c	8.7 ± 1.1e
Emamectin	2.7 ± 0.2d	1.7 ± 0.1f	0.7 ± 0.2e	0.6 ± 0.2e	0.7 ± 0.4f	0.9 ± 0.5gh
Imidacloprid + spinosad	3.3 ± 0.2bc	2.6 ± 0.2cd	2.6 ± 0.3c	1.5 ± 0.5c	2.3 ± 0.8d	2.6 ± 1.0fg
Imidacloprid + *B. bassiana*	3.7 ± 0.1ab	3.3 ± 0.2ab	3.3 ± 0.3b	6.6 ± 0.8b	9.3 ± 0.8bc	10.6 ± 0.8cd
Imidacloprid + emamectin	3.2 ± 0.2c	1.9 ± 0.2ef	1.9 ± 0.3d	0.5 ± 0.2d	0.5 ± 0.3f	0.7 ± 0.4h
Acetamiprid + spinosad	3.0 ± 0.2c	2.3 ± 0.3de	2.3 ± 0.2cd	1.2 ± 0.2cd	1.7 ± 0.6de	2.1 ± 0.8f
Acetamiprid + *B. bassiana*	3.6 ± 0.1b	3.0 ± 0.2bc	3.0 ± 0.2b	5.6 ± 0.9b	7.2 ± 1.2c	8.6 ± 1.4de
Acetamiprid + emamectin	2.6 ± 0.2d	1.8 ± 0.3ef	1.8 ± 0.3d	0.7 ± 0.3d	0.8 ± 0.3ef	1.2 ± 0.5fgh
F (df), *P* treatment	11.9 (12, 143), < 0.0001	21.9 (12, 143), < 0.0001	30.6 (12, 143), < 0.0001	34.9 (12, 143), < 0.0001	41.5 (12, 143), < 0.0001	46.2 (12, 143), < 0.0001
F (df), *P* treatment (Welch)	—	32.9 (12, 55.3), < 0.0001	35.4 (12, 55.5), < 0.0001	50.6 (12, 55.2), < 0.0001	85.5 (12, 55.3), < 0.0001	90.0 (12, 55.3), < 0.0001

Means in the same column followed by a common letter are not significantly different ($P = 0.05$, Tukey's HSD test).

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spider mite numbers. In contrast, both spinosad and emamectin either alone or in combinations with imidacloprid and acetamiprid resulted in significantly fewer mites than the control. *Beauveria bassiana* was the least effective in controlling mite populations.

Concerning the fecundity experiment, Table 3 displays the observations taken at various DAT on the numbers of eggs laid by spider mites on rose leaves treated with various insecticides and their combinations. Imidacloprid drench and foliar treatments showed significantly greater numbers of eggs than controls during the early observation days up to 9 DAT for drench and up to 7 DAT for foliar treatments. The acetamiprid treatment did not show any significant difference in egg numbers at all DAT compared with the controls. Spinosad, emamectin, and *B. bassiana* alone or in combinations showed a significant reduction in egg numbers in comparison with controls at almost all observation days.

In the preference and non-preference test, the behavioral response (food choice) of adult spider mites was studied by giving them a choice of insecticide-treated leaves vs. untreated leaves and observing the length of time the mites would abide by their initial choice. As displayed in Fig. 1, the number of mites that reached acetamiprid-treated leaf discs vs. untreated leaf discs was significantly less at 1 DAT (χ^2 = 22.9, P < 0.0001), but did not differ significantly thereafter at 3 DAT. On imidacloprid drench-treated leaves, significantly more spider-mites were observed at 5 DAT (χ^2 = 4.6, P = 0.0203) and 7 DAT (χ^2 = 7.9, P = 0.0048) than on the untreated control leaf discs. Similarly, on foliar applied imidacloprid-treated leaf discs, there were significantly more mites at 5 DAT (χ^2 = 5.7, P = 0.0165) and 7 DAT (χ^2 = 7.6, P = 0.0059) than on untreated leaf discs. Also, at 1 DAT on foliar applied imidacloprid-treated leaf discs, there were significantly fewer mites than on the untreated leaf discs (χ^2 = 15.5, P < 0.0001).

Discussion

The increase in mite numbers occurred sooner on leaves of rose plants to which imidacloprid had been applied by drench than on those to which either imidacloprid or acetamiprid had been applied by foliar spray. Probably the increase in fecundity occurred sooner on drench-treated plants because the imidacloprid after being absorbed by the roots reached all of the cells of the plant directly and swiftly via the vascular.

My results with spinosad (Table 2) are in agreement with those of Villanueva & Walgenbach (2006), who placed *T. urticae* nymphs individually on bean, *Phaseolus vulgaris* L. (Fabales: Fabaceae), leaf discs treated with spinosad at 25, 55, 121, and 266 ppm and observed that very few (< 15%) nymphs completed development and that significantly higher mortality and lower oviposition rates were obtained from adult females at all the concentrations tested as compared with the control. Moreover, spinosad was highly persistent in quantities as low as 1 mg per plant, when applied to the roots of tomato plants in rock wool, and gave significant and long-lasting control of spider mites (Van Leeuwen et al. 2005). When spinosad at 20, 25, 30, 35, and 40 mg/L, abamectin at 0.125, 0.25, 0.5, 1.0, and 2.5 mg/L, and combinations of both were tested by direct spraying of leaf discs against *T. urticae*, adulticidal and ovicidal effects were reported with both insecticides and combined applications, although spinosad caused more harm to the pest than abamectin (Ismail et al. 2007).

The data obtained with the preference and non-preference test surprisingly indicated that within 5 to 7 d after applying the imidacloprid drench to the rose plants, imidacloprid changed some attributes of plant that made them preferred over untreated plants. These results coincide with the fecundity observations (Table 2), which showed that up to 7 DAT (foliar) or 9 DAT (drench), the mites laid greater numbers of eggs in the imidacloprid treatments compared with the control.

These imidacloprid-induced changes were evident in the report by James & Price (2002) as they first reported the effect of imidacloprid on fecundity of *T. urticae* that had fed on leaf discs of a bean plant that was exposed systemically to imidacloprid. Imidacloprid-treated *T. urticae* produced 10 to 26% more eggs during the first 12 d of adult life and 19 to 23% more during adulthood compared with a water-only treatment (James & Price 2002). Szczepaniec et al. (2011) reported that the fecundity of *Tetranychus schoenei* McGregor (Prostigmata: Tetranychidae) that consumed leaves from treated elms increased by nearly 40% compared with females feeding on untreated foliage. This effect, however, was only present when spider mites consumed leaves from treated elms. Interestingly, Szczepaniec et al. (2011) observed that higher reproduction of *T. schoenei* was accompanied by measurable changes in plant physiology demonstrated by a nearly 20% increase in the area of elm leaves. Also, Szczepaniec & Raupp (2012) found that imidacloprid significantly increased the fecundity

Table 3. Mean (± SE) number of eggs laid in various treatments at various days after treatment (DAT). Eggs were laid and mites were kept in a different Petri dish after each observation. Data were analyzed by ANOVA (JMP SAS Institute 2011).

Treatment	1 DAT	3 DAT	5 DAT	7 DAT	9 DAT	11 DAT
Control	4.3 ± 0.4ab	9.1 ± 0.5b	10.7 ± 0.7c	13.7 ± 0.9bc	13.1 ± 0.9b	14.0 ± 0.9ab
Imidacloprid drench	9.4 ± 0.9a	16.4 ± 0.8a	22.3 ± 0.9a	17.8 ± 1.4a	15.6 ± 1.1a	15.2 ± 1.2ab
Imidacloprid foliar	9.1 ± 0.4a	17.3 ± 0.5a	23.6 ± 1.2a	16.8 ± 1.5ab	17.8 ± 1.3ab	16.2 ± 0.8a
Acetamiprid	5.6 ± 0.3ab	11.0 ± 0.6bc	10.5 ± 0.8bc	12.4 ± 0.8c	13.5 ± 0.7b	12.1 ± 0.7bc
Spinosad	2.4 ± 0.3cd	1.5 ± 0.2f	2.1 ± 0.3ef	2.9 ± 0.3de	2.3 ± 0.3d	2.5 ± 0.3e
Beauveria bassiana	3.4 ± 0.3bcd	4.1 ± 0.4de	4.6 ± 0.5de	5.3 ± 0.3de	8.1 ± 1.0c	8.1 ± 0.6d
Emamectin	2.3 ± 0.3cd	2.3 ± 0.2ef	1.7 ± 0.2f	2.6 ± 0.3de	2.9 ± 0.3d	3.1 ± 0.3e
Imidacloprid + spinosad	1.9 ± 0.2d	3.1 ± 0.3def	2.1 ± 0.3ef	2.5 ± 0.2e	2.7 ± 0.8d	3.1 ± 0.8e
Imidacloprid + *B. bassiana*	3.1 ± 0.2bcd	4.5 ± 0.5d	5.2 ± 0.5d	6.0 ± 0.3d	9.4 ± 0.7c	9.2 ± 0.6cd
Imidacloprid + emamectin	1.9 ± 0.2d	2.0 ± 0.2f	1.9 ± 0.2ef	2.3 ± 1.4e	3.3 ± 0.3d	3.2 ± 0.2e
Acetamiprid + spinosad	2.4 ± 0.3bc	2.3 ± 0.2def	1.8 ± 0.5d	2.5 ± 0.5de	2.9 ± 0.6c	3.3 ± 0.5d
Acetamiprid + *B. bassiana*	3.8 ± 0.4cd	3.0 ± 0.4ef	5.2 ± 0.2f	5.6 ± 0.3e	8.1 ± 0.5d	7.8 ± 0.4e
Acetamiprid + emamectin	2.5 ± 0.2cd	2.0 ± 0.2f	1.7 ± 0.3f	2.4 ± 0.3e	3.7 ± 0.5d	3.2 ± 0.3e
F (df),	57.8 (12, 143),	188 (12, 143),	182.5 (12, 143),	67.8 (12, 143),	53.1 (12, 143),	59.7 (12, 143),
P treatment	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F (df),	53.9 (12, 55.5),	119.3 (12, 55.3),	91.9 (12, 55.3),	45.1 (12, 55.4),	50.2 (12, 55.2),	51.6 (12, 55.3),
P treatment (Welch)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means in the same column followed by a common letter are not significantly different (P = 0.05, Tukey's HSD test).

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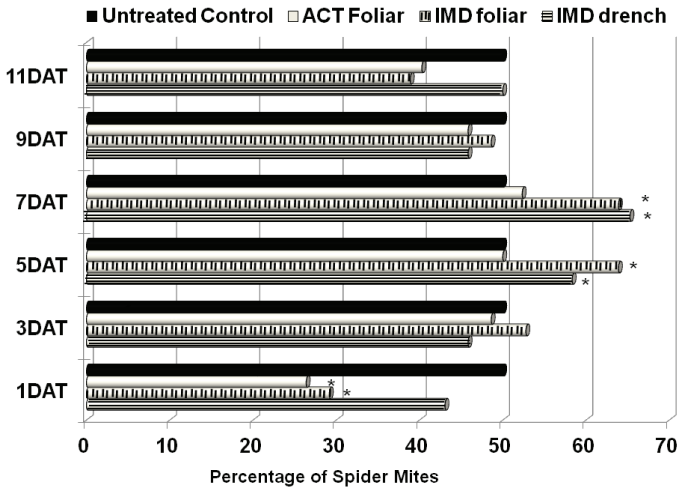


Fig. 1. Preference and non-preference test for spider mites by providing imidacloprid (IMD)-or acetamiprid (ACT)-treated and untreated rose leaves as 2 choices at different days after treatment (DAT) and observing the percentage of spider mites reaching a specific choice. Asterisk indicates significant difference between treatment and untreated control ($P = 0.05$, χ^2 goodness of fit).

of *Eurytetranychus buxi* (Garman) (Prostigmata: Tetranychidae) feeding on imidacloprid-treated shrubs and boxwoods. Spider mites that fed on foliage from boxwoods treated with imidacloprid laid more eggs, whereas the insecticide had no effect on reproductive performance of mites when it was applied as a topical spray (Szczepaniec & Raupp 2012). Imidacloprid-induced outbreaks of spider mites on roses, *Rosa* sp., also were associated with greater leaf area, increased chlorophyll indices, and elevated nitrogen content (Gupta & Krischik 2007). Thus, there is growing evidence that imidacloprid affects spider mites by improving the quality of plants as hosts of this herbivore.

Because of the complex effects of some insecticides, such as the neonicotinoids, integrated control and resistance management strategies should be implemented for the entire pest complex of roses. Based on the results of this study, I have concluded that spinosad and emamectin in combinations with neonicotinoids can be incorporated in the IPM of roses. Limiting the neonicotinoid use will have the dual benefit of delaying the onset of resistance and reducing the probability of mite outbreaks.

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