Research Article

Evaluation of Five Different Botanical Extracts Against Some Pests and Predators in Laboratory Conditions

Bilal Saeed Khan[1]*, Muhammad Aneeb Shahzad[1], Muhammad Irfan Ashraf[2], Zahid Mahmood Sarwar[3], Muhammad Farooq[4] and Awias Rasool[5]

[1]Department of Entomology, Faculty of Agriculture, University of Agriculture, Faisalabad, Pakistan; [2]Institute of Horticultural Sciences, UAF, Pakistan; [3]Department of Entomology, BZU, Multan, Pakistan; [4]Citrus Research Institute, Sargodha, Pakistan; [5]National Agricultural Research Centre (NARC), Islamabad, Pakistan.

Abstract | The current study was conducted to evaluate the toxicity and repellency of leaf aqueous extracts i.e., *Melia azedarach* (Bakain), *Nicotiana tabacum* L. (Tobacco), *Eucalyptus globulus* Labill (Eucalyptus), *Moringa oleifera* L. (Moringa) and *Mentha piperita* L. (Peppermint) against two major sucking pests i.e. aphid (*Sitobion avenae* F.) and two-spotted spider mites (*Tetranychus urticae* Koch) and their natural enemies such as ladybird beetle (*Coccinella septempunctata* Linnaeus) and predatory mite (*Amblyseius andersoni* Chant). The leaf dip method was used to conduct bioassay and this experiment was performed under Complete Randomized Design (CRD) with three replications. The results showed that *N. tabacum* extract was more toxic to aphids, two-spotted spider mites, ladybird beetles and predatory mites and caused 80.82, 81.49, 39.77 and 51.55% mortalities, respectively, after 96 hours. *M. azedarach* extract showed the second highest mortalities i.e., 73.85% in aphids, 70.54% in two-spotted spider mites, 31.05% in ladybird beetles and 46.49% of predatory mites. Mortality trend observed in other aqueous extracts was eucalyptus > moringa > peppermint. The repellent effect of these extracts was also evaluated and observed after 12 and 24 hours. *N. tabacum* extract caused 80.55 and 86.88% repellencies against aphid and two-spotted spider mites, while 68.55 and 74.34% against ladybird beetle and predatory mites, followed by bakain > peppermint > moringa > eucalyptus. The results revealed that all these leaf aqueous extracts can be used effectively against aphids and two-spotted spider mites, but we have to be careful with their slightly toxic effects on predators.

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***Correspondence** | Bilal Saeed Khan, Department of Entomology, Faculty of Agriculture, University of Agriculture, Faisalabad, Pakistan; **Email:** bilalentomologyuaf@gmail.com
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Introduction

Botanical pesticides are ideal for organic farming since they are less dangerous to humans, disintegrate quickly, and have fewer residual effects with the least adverse environmental impact than chemical insecticides (Akbar *et al*., 2021). Pyrethrum, rotenone, hellebore, nicotine, veratrum alkaloids, and quassin were used before the advent of synthetic insecticides. Over 2400 plants have been identified with pest control properties. Botanical insecticides keep attracting the attention of small farmers worldwide as they are considered a suitable alternative to synthetic insecticides (Pavela, 2016). Farmers have employed a range of plants to create bioinsecticides. Neem (*Azadirachta indica*), calotropis (*Calotropis procera*), lemongrass (*Cymbopogon citratus*), lemon leaves (*Citrus limon*), lantana (*Lantana camara*), and moringa (*Moringa oleifera*) are a few examples of such plants (Majeed *et al*., 2018). All these compounds are pest management agents acting as insect growth regulators and feeding deterrents. Problems associated with synthetic insecticides led researchers to look for natural plant protection compounds, such as botanical insecticides, as they are practical and specifically target plants' natural enemies (Isman, 2006).

Over the decades, pesticides have been widely applied as a standard practice to control agricultural pests. Their constant use has caused a selection of resistance to farm pests and environmental pollution with adverse side effects on human health and non-target arthropods (Shah *et al*., 2017). According to Rani *et al*. (2021), pesticides produce severe diseases and disorders in agricultural workers when they come in contact with skin or other means. Among the diverse, environmental-friendly and safe strategies for pest management, biopesticides represent one of the best alternatives to chemicals (Copping and Menn, 2000). Botanical insecticides are considered as a source of eco-friendly and sustainable crop production. However, technical obstacles and challenges have limited their effectiveness to date (Nielsen *et al*., 2008). Current research into biopesticides focuses on the improvement of their action spectra, including mechanisms to replace the use of chemical pesticides in IPM plans (Nawaz *et al*., 2016). Among the South Asian countries, Pakistan ranks second in the overall consumption of pesticides, and the primary use of these pesticides is in the agriculture sector (Waheed *et al*., 2017). Pakistan has a high consumption of pesticides (Majeed *et al*., 2020), with an alarming increase of 1169% in the last two decades, with more than ten sprays per crop season (Khan *et al*., 2020).

The bio-potential of many species of plants has been studied by several workers globally against aphid species. The studies so far indicate the effectiveness of peppermint extracts in combating certain aphid species, e.g., peach-potato aphid (Ikeura *et al*., 2012). Tobacco extract effectively controlled the aphid population, followed by neem extract, whereas garlic extract remained the least effective against aphids (Sohail *et al*., 2012). Plant extracts such as *Azadirachta indica* A., *Eucalyptus globulus* L., *Ocimum basilicum* L., etc., could control the population of severe pests like aphids and mealybugs in an environmentally friendly way (Singh *et al*., 2012). The cost-benefit ratio of botanical pesticides might be comparable to that of chemical insecticides and might be used against aphids (Aziz *et al*., 2013). Excessive use of insecticides has jeopardized the environment and seriously threatened human health (Tayyab *et al*., 2021). Therefore, the current study aims at:

Laboratory evaluation of five different botanicals against major sucking pests, i.e., aphid and two-spotted spider mites, and their impacts on their natural enemies, i.e., ladybird beetle and predatory mites, to prove their toxicity and repellent effects.

Materials and Methods

Preparation of botanical extracts

Aqueous extracts were prepared using 250 grams of leaves (shade dried), making its powder with an electric grinder and then dissolving it in 500 ml of water on a hot plate with a magnetic stirrer for 24-36 hours. Aqueous extracts were filtered by using the Whatman no 1 filter paper. The magnetic stirrer was set at 100 rpm/ minute at 40°C temperature, and then an aluminum foil was placed on it to prevent contamination and placed in the refrigerator. This aqueous extract was considered 100%. 10, 5, 2.5 and 1.25% concentrations of each botanical extracts were prepared by serial dilution. Data regarding the percentage mortality of the insect population was corrected by using Henderson and Tilton's (1955) formula:

$$\%\ \text{Mortality} = \left(1 - \frac{N\ in\ Control\ before\ Treatment\ \times N\ in\ Treatment\ after\ Treatment}{N\ in\ Control\ after\ Treatment\ \times N\ in\ Treatment\ before\ Treatment}\right) \times 100$$

Where; N is donated by the number of specimens.

The repellent effect of the botanical extracts against aphids, ladybird beetles, two-spotted spider mites and predatory mites was calculated as they moved away from the corresponding treated surface of the leaves. Percent. repellency was calculated using the repellency percentage formula where Ta= Control and Tb= Treatment (Koagan and Goeden, 1970):

$$\% \text{ Repellency } = \left[\left(\frac{Ta - Tb}{Ta}\right) \times 100\right]$$

Insect culture and bioassay

To conduct the bioassay against adults of aphid and ladybird beetle, partially wet tissue was placed in a Petri dish. The infested leaves of brinjal, chili, and tomato from the field area having adults of aphid, two-spotted spider mite, ladybird beetle, and predatory mite were brought to the laboratory in polythene bags. Pests were kept under room conditions at 27±2°C to ensure the continuous availability of pests during the experiment. Predators were reared in bio-control cages under controlled room temperature conditions 27±2°C and 75±5% relative humidity. This experiment was conducted under Complete Randomized Design (CRD) using three replications. Fresh leaves of tomato were washed, dried, distilled and placed in a cell arena. A leaf dip method (IRAC method 7) was employed for aqueous bioassay with four serial dilutions, i.e., 10, 5, 2.5 and 1.25%. The treated leaves with varying concentrations of botanical extracts were placed on wet tissues. Mite cells were prepared to conduct the bioassay against two-spotted spider mites and predatory mites. Circular-shaped leaves were designed with a diameter of 30 mm and were placed on cotton. The cotton was partially soaked in water and was used in a Petri dish to avoid mite escape. A total thirty adults of each pest and predator, ten for each replication adults, were released in these different cells containing different serial dilutions. The mites were allowed to acclimate for about 30 minutes. Data was collected after 24, 46, 72 and 96 hours of bioassay.

The repellent effects of five different botanicals were recorded using a treated surface and a controlled surface of leaf discs in a petri dish. Leaves were cut circularly with a diameter of 2 inches and were placed on cotton soaked in water and a strip of filter paper was placed between them. Two circular-shaped leaves were placed on opposite sides of the petri dish. One was dipped in botanical solution and the other in water for 10 seconds. Ten individuals of adult insects and adult mites were placed on filter paper strips with the help of a camel hair brush, and their movement was recorded after 12 and 24 hours.

Statistical analysis

Data regarding corrected mortality and repellency of adult spider mites, predatory mites, aphids, and ladybird beetle was subjected to Minitab 18 Statistical software for one-way analysis of variance (ANOVA) at 95% level of significance where α=0.05, to find out the mean values for different treatments of each aqueous extract against pest and predator. The means of significant treatment were compared using Tuckey's HSD test.

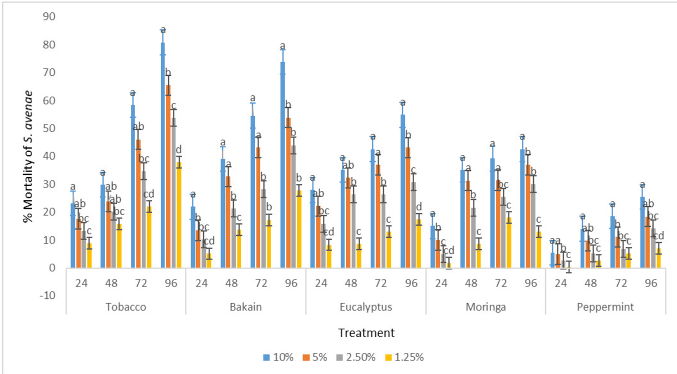

Figure 1: *Effect of botanicals with different concentrations on the percent mortality against Sitobion avenae (Aphid) after 24-96 hours.*

Results and Discussions

Mortality of pests

The results have showed that the mortality of *S. avenae* F. (Figure 1) was significantly (P≤0.05) affected by increasing the concentration. The mean value of the data revealed that maximum mortality (i.e. 80.82±1.52) by 10% *N. tabacum* leaf extract as compared to findings of Abid (2005) determined that tobacco, compared to other plant extracts like neem, datura, and onion, had a higher potential for toxicity against wheat aphid (*Schizaphus graminum*) causing 97.15% mortality, followed by neem leaf extract (81.21%) and minimum mortality (i.e. 25.49±1.96) was observed in case of 10% *M. piperita* L. leaf extract is familiar with Biniaś *et al.* (2017) which also found same % mortality by 10% *M. piperita* L. extract against black bean aphids. Other botanicals were also influential in causing the mortality of *S. avenae,* as *M. azedarach* leaf extract caused 79.85±1.79 mortality and was also remained effective against the aphid population. *M. oleifera* and *E. globulus* leaf extract remained medium effective against the aphid population, causing 54.9±1.77 and 42.59±1.74 mortalities, respectively. Farooq *et al.* (2016) also observed a similar trend of results against *S. avenae* in field condition but there is a difference in methodology. Results from the second experiment regarding mortality of (*T. urticae*) two-spotted spider mite (Figure 2) showed that 10% *N. tabacum* leaf extract caused 81.49±1.03 mortality, similar to the results of Akyazi *et al.* (2018) found tobacco leaf extract most lethal against *T. urticae*. *M. azedarach* leaf extract at maximum concentration (10%) caused 76.21±4.36 mortality. Current finding of (*M. azedarach*) could be correlated with results of Wakgari and Yigezu (2018) as they recorded 70.55% mortality of two-spotted spider mites. *E. globulus* leaf extract caused 56.56±0.627 mortality, which contradicts the study of Hamed *et al.* (2021) due to the difference in methodology. *M. oleifera* leaf extract caused 43.17±1.12, while the lowest value of mortality was observed by *M. piperita* L. leaf extract at 28.69±1.73.

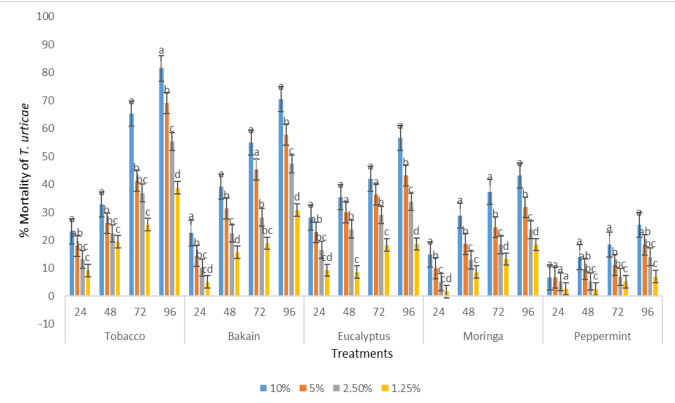

Figure 2: *Effect of botanicals with different concentrations on the percent mortality against Tetranychus urticae (Two-spotted spider mite) after 24-96 hours.*

Mortality of predators

Results have revealed that maximum mortality (39.77±0.895) against *C. septempunctata* (Figure 3) was observed by *N. tabacum* leaf extract using 10% concentration as familiar with results of Abid (2005) and Kunbhar *et al.* (2018) on mortality of ladybird beetle in which neem and tobacco showed the highest toxicity levels for ladybird beetle. Minimum mortality (18.24±1.62) was observed in this bioassay by 10% concentration of *M. piperita*. L leaf extract. Other botanicals also proved their toxicity in causing mortality against *C. septempunctata*, as 10% concentration of *M. azedarach* leaf extract caused 31.05±0.527 mortality after 96 hours of study. Highest concentration (10%) of *E. globulus* leaf extract caused 25.69±0.546 mortality, which is somewhat similar to the results of Gemmeda and Ayalew (2015), who found toxic effects of eucalyptus against ladybird beetle while *M. oleifera* leaf extract stood medium effective against *C. septempunctata* population causing 28.52±0.645 mortality. The results from the second experiment regarding mortality of *A. andersoni* (Figure 4) showed that 10% concentration of *N. tabacum* leaf extract caused 51.55±1.19 mortality, followed by 10% of *M. azedarach* leaf extract 46.49±0.724 mortality as compared to work of Castagnoli *et al.* (2002) which observed 46% mortality of predatory mites by using bakain leaf extracts. *E. globulus* leaf extract caused 36.00±0.294 mortality, *M. oleifera* leaf extract 24.38±0.546 while the most negligible value of mortality was observed by 10% concentration of *M. piperita* L. that is 16.47±0.612.

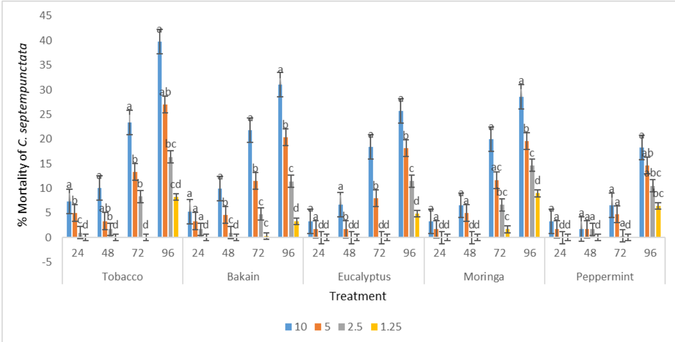

Figure 3: *Effect of botanicals with different concentrations on the percent mortality against Coccinella septempunctata (Ladybird beetle) after 24-96 hours.*

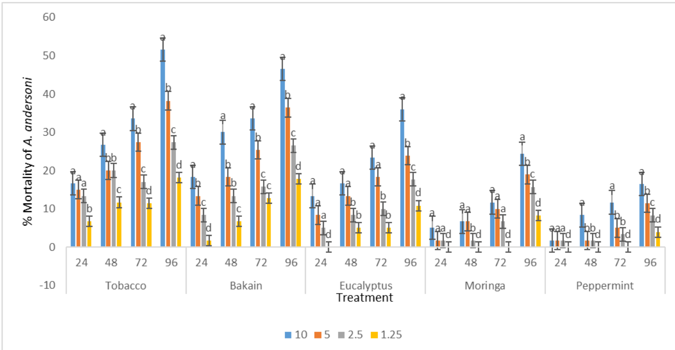

Figure 4: *Effect of botanicals with different concentrations on the percent mortality against Amblyseius andersoni (Predatory mite) after 24-96 hours.*

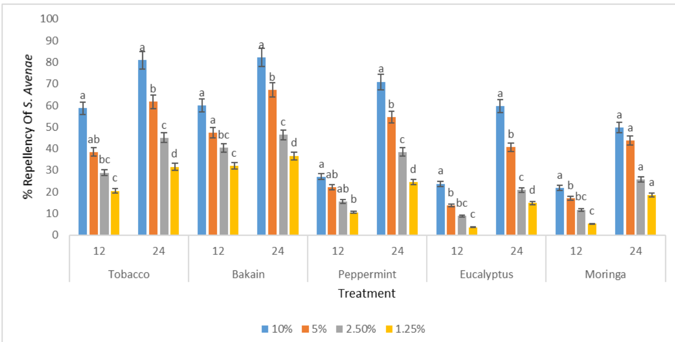

Figure 5: *Effect of botanicals with different concentrations on the percent repellency against Sitobion avenae (Aphid) after 12 and 24 hours.*

Repellency of pests

Conclusions have shown a significant variation (P≤0.05) by increasing the concentration. Maximum repellency of aphid and two-spotted spider mites (Figures 5 and 6) against maximum concentration (10%) of *N. tabacum* leaf extracts. Repellent effects of tobacco are proved by Weber *et al.* (2019). *M. azedarach* leaf extracts at 10% concentration displayed (60%, 80.33%, 58.66%, 76.88% and 60.63%, 86.67%, 55.44%, 82.88%) repellency as somewhat similar to the results of Sharma *et al.* (2010) who followed 83.3% and 93.33% repellency in two-spotted spider mites by using bakain extracts. Sukrutha *et al.* (2023) also proved *Melia azedarach* is the best repellent against different kinds of pests. *M. piperita* leaf extracts of 10% stand second to them, showing (31.66, 65.10 and 31.77, 63.55) % repellency is in-line with the study of Wubie *et al.* (2014), which observed 65.1% repellency in aphids by using peppermint leaf aqueous extracts while results regarding repellent effects of peppermint against two-spotted spider mites are contradictory with Tehrani *et al.* (2022) due to difference in methodology. Maximum concentration (10%) of *E. globulus and M. oleifera* also shown repellent effects such as (18.33%, 46.44%, 24.88%, 52.1% and 21.67%, 53.11%, 26.1%, 45.43%), respectively, as compared to findings of Solangi *et al.* (2011) observed repellent effects of *E. globulus* against pests and Ogbonna *et al.* (2021) which observe 53.11% repellency by *M. oleifera* under controlled conditions against aphids.

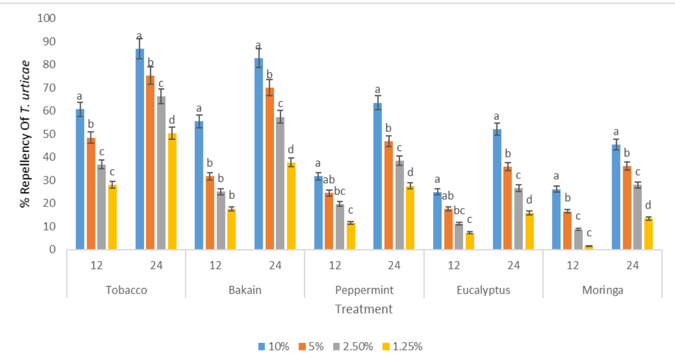

Figure 6: *Effect of botanicals with different concentrations on the percent repellency against Tetranychus urticae (Two-spotted spider mites) after 12 and 24 hours.*

Repellency of predators

Results revealed that *N. tabacum* and *M. azedarach* leaf extracts have the highest level of repellent effects against ladybird beetle and predatory mite (Figures 7 and 8) such as (50%, 68.53%, 60%, 78.53% and 36.67%, 75.55%, 38.33%, 76.83%), respectively is also proved by Ali *et al.* (2017) observed repellent effects of moringa and tobacco against stored grain pests. Among all botanicals used, *M. piperita* leaf extract showed (26.67, 63.73, 31.67, 65.10) % repellency. Under controlled circumstances, *E. globulus* and *M. oleifera* leaf extracts also demonstrated a repellent effect i.e., (16.67% 45.77%, 18.33%, 46.43% and 20.67%, 52.43%, 23.67%, 53.11%). Sheikh *et al.* (2021) also found *E. globulus* extract having better repellent properties while Mohammad and Iddriss (2023) evidenced repellent properties found in *Moringa oleifera* leaf extracts.

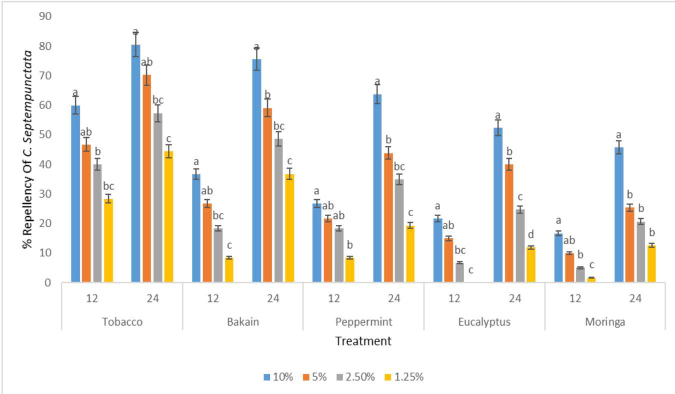

Figure 7: *Effect of botanicals with different concentrations on the percent repellency against Coccinella septempunctata (Ladybird beetle) after 12 and 24 hours.*

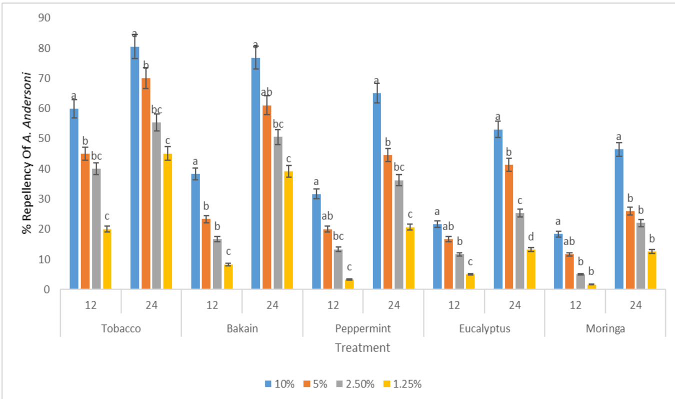

Figure 8: *Effect of botanicals with different concentrations on the percent repellency against Amblyseius andersoni (Predatory mite) after 12 and 24 hours.*

Conclusions and Recommendations

All botanicals in the laboratory showed significant toxic effects against both pest and predator species. Among botanicals, *N. tabacum* and *M. azedarach* leaf aqueous extracts have shown high toxicity against pests and predators, even when used in lower concentrations. Similarly, *E. globulus* and *M. oleifera* demonstrated toxic and repellent effects against the pest population. In a nutshell, botanicals could be an alternate means against pest populations wildly sucking species. It is assumed that these botanicals would also have a toxic and repellent effect against these insect pest species in field conditions.

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Novelty Statement

The study demonstrated the impact of different botanicals against some sucking pests and predators to prove their toxicity and repellency and these extracts will certainly be used in future applications regarding pest control.

Author's Contribution

Bilal Saeed Khan: Conceived the idea and overall management of the article.
Muhammad Aneeb Shahzad: Wrote abstract, methodology and data collection.
Muhammad Irfan Ashraf: Technical input at every step.
Zahid Mahmood Sarwar: Wrote discussion and cnclusion.
Muhammad Farooq: Data entry, analysis and references.
Awias Rasool: Indroduction, proof reading and reference correction.

Conflict of interest
The authors have declared no conflict of interest.

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