

Evaluating the Role of Potassium Silicate and Bordeaux Mixture in Enhancing Closer Insecticide Efficacy Against Woolly Apple Aphid (*Eriosoma lanigerum*)

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Abstract. This study aimed to field test the effectiveness of Closer insecticide with two adjuvants, potassium silicate and Bordeaux mixture, against woolly apple aphid (*Eriosoma lanigerum* Hausmann) on two local cultivars (Sharabi and Ibrahim) of apple. grown in Al-Alam District, Salah al-Din Governorate, Iraq. A field experiment was laid out in a randomised complete block design (RCBD) with three replications for each cultivar. The following six treatments were evaluated: Control (Water Only), Potassium Silicate, Bordeaux Mixture, Closer insecticide (sulfoxaflor), Closer + Potassium Silicate and Closer + Bordeaux Mixture. Colony counts were done at 3, 7, 14 and 21 days after treatment. The treatment Closer + Bordeaux mixture resulted in the greatest reduction of waxy colony density at 21 days with 94.26% and 92.76% for Sharabi and Ibrahim cultivars, respectively. Closer + potassium silicate was the next best combination with a reduction of 88.66% and 87.58% respectively as compared to 79.78% for Closer alone. The Sharabi cultivar always gave a higher response level than the cultivar called Ibrahim. 99.83% sequence identity was obtained with *Eriosoma lanigerum* voucher CNC#HEM028323 (KR043025.1) using the mitochondrial COX1 gene; while the Iraqi isolate (Aw.) was identical. The sequence obtained from J-3) was and is deposited in GenBank with accession number OP776810.1. The findings indicate that the use of Closer in conjunction with Bordeaux mixture or potassium silicate was effective as strategy in the integrated pest management (IPM) programme to control woolly apple aphid (WAA) in the apple orchard.

Keywords: *Eriosoma lanigerum*, Woolly Apple Aphid, Sulfoxaflor, Closer, Potassium Silicate, Bordeaux Mixture.

INTRODUCTION

Apple (*Malus domestica* Borkh.) is one of the most economically and nutritionally important crops of temperate regions of the world. An important role of its fruits is that they contribute high sugar, organic acid, dietary fibre, vitamin and phenolic content, which is significant in several food processing industries [10]. The orchards be come more exposed to insect pests and the

use of chemical insecticides has grown, making sustainable pest management a key challenge.

Among the most economically damaging pests of apple trees worldwide is the woolly apple aphid, *Eriosoma lanigerum* Hausmann. It is a cosmopolitan species that infests the aerial parts of apple trees, feeding on the phloem leading to galls, deformities and a reduction of vegetative growth and

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productivity. The sooty mould formed by the colonies of aphids on the honeydew affects plant photosynthetic ability and fruit quality [5, 16]. Aphids are often found in the bark crevices and sheltered microhabitats within colonies which further hinders chemical control [1], and the protective waxy woolly covering over colonies makes it difficult for insecticides to penetrate.

Closer insecticide that contains sulfoxaflor is a modern and effective measure to control piercing-sucking insects. Its mode of action is to bind the nicotinic acetylcholine receptors, which is the basis of its ability to inhibit the nervous system of insects and lead to death [19]. Sulfoxaflor is effective against many species of aphids but the waxy cover on woolly apple aphid colonies might reduce its efficacy, and evaluation of appropriate adjuvants to enhance field performance is warranted [14] Resistance to chemical insecticides has also been reported as a common issue in aphid populations, further emphasizing the importance of adopting more integrated management practices, particularly when using chemical-based tactics, as part of an IPM program [4].

There has been a great deal of interest in potassium silicate as an adjuvant and as an elicitor of plant resistance. The silicon Silicon has been widely reported to enhance plant resistance against sap-sucking insects.

Silicon deposition in plant tissues reinforces cell walls and activates defence pathways, resulting in reduced aphid feeding, reproduction, and colonisation [8,9]. Accordingly, several studies have demonstrated that silicon application decreases aphid colony density and feeding efficiency [7,13,15], and potassium silicate has been shown to reduce aphid populations under field conditions in Iraq [18]. In addition, Bordeaux mixture has been reported to modify the chemical properties of the bark surface, making bark crevices less favourable for woolly apple aphid colonisation and potentially improving the effectiveness of subsequent insecticide applications.

Although the adjuvants are relevant to control the woolly apple aphid in combination with modern systemic insecticides, few studies have been conducted to evaluate the combinations of these adjuvants with modern systemic insecticides to control the woolly apple aphid against locally important cultivars of apple. Hence, this study aimed to assess the efficacy of potassium silicate and Bordeaux mixture on the efficacy of the spray Closer in controlling the *E. lanigerum* waxy colony density under field conditions on Sharabi and Ibrahimi apple cultivars and to provide evidence for the use of these materials in IPM strategies.

MATERIALS AND METHODS

Study site

The experiment was carried out in the commercial apple orchard of Al-Alam District, Salah al-Din Governorate during the 2024-2025 growing season. Trees of two cultivars – Sharabi and Ibrahimi – were naturally infested in the orchard (separate fields). The orchard area was about 4000 m² and split into two equal parts between the two cultivars. The Sharabi field was 40m by 50m (2,000m²) with trees planted at 4m by 5m, and the Ibrahimi field was the same size (2,000m²), with the same tree planting spacing (4m by 5m). All orchard practices were common in both fields, and applied by

the same treatment throughout the season, such as regular tillage and conventional irrigation. Experimental trees were selected from both fields to minimise replicate variation and ensure a uniform experimental setup, using uniformity of age (10–12 years), canopy size and initial infestation level.

Morphological identification of the pest

The characteristic morphologic features of *Eriosoma lanigerum* Hausmann (white waxy woolly colonies covering branches, shoots and tissues of trunk; sap-feeding leads to galls and deformities typical for the species) were used for field identification of the pest. Figure (1)



Figure (1). Symptoms of woolly apple aphid infestation on apple trees before treatment application, showing dense waxy white masses and aphid colonies on trunks and branches.

Molecular identification

Molecular identification of the pest was performed according to the method of [2] using the mitochondrial cytochrome c oxidase subunit I (COX1) gene. Samples were collected from infected

trees and preserved in 95% ethanol until DNA extraction.

Genomic DNA was extracted using the G-spin DNA Extraction Kit (Intron Biotechnology, cat. no. 17045) following the manufacturer's instructions. Approximately 25 mg of insect tissue was homogenized in Buffer CL containing proteinase K

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and RNase A, followed by lysis with Buffer BL, centrifugation, purification through a spin column with Buffers WA and WB, and elution in Buffer CE.

The COX1 fragment was amplified by PCR using the universal primers of Folmer et al. (1994): forward 5'-GGTCAACAAATCATAAAGATATTG-3' and reverse 5'-TAAACTTCAGGGTGACCAAAAAATCA-3', producing an amplicon of approximately 720 bp.

Experimental design and treatments

The experiment design was randomised complete block design (RCBD) replicated thrice in each cultivar. The 72 trees were divided into six treatments: (1) control (distilled water); (2) potassium silicate; (3) Bordeaux mixture; (4) Closer insecticide (sulfoxaflor); (5) Closer + potassium silicate; and (6) Closer + Bordeaux mixture.

Preparation of treatments

Potassium silicate (35% SiO₂ ; 12% K₂ O) was prepared as a foliar spray at a concentration of 1 mL L⁻¹ in distilled water. The insecticide was formulated according to the recommended field rate of 35 mL/100 L (closer) of water. Bordeaux mixture was made by dissolving 1 kg of copper sulphate and 2 kg of hydrated lime in 10 L of water just prior to application.

Treatment application

Hand-held sprayer was used to spray potassium silicate solution to run-off on foliage with special consideration of the aphid infested areas. Further insecticide application was done with a knapsack sprayer ensuring good and even coverage of all foliage. Trunk and main-branch washing with Bordeaux mixture was used to treat areas of bark crevices and protected surfaces where colonies of woolly aphids were found. For combined treatments (C), Closer

PCR reactions (25 µl) contained 5 µl Maxime PCR PreMix (i-Taq; Intron Biotechnology; cat. no. 25025), 1 µl of each primer (10 pmol/µl), 1.5 µl template DNA, and nuclease-free water. Thermal cycling consisted of an initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 45 s, 58°C for 45 s, and 72°C for 45 s; followed by a final extension at 72°C for 7 min. PCR products were separated on 2% agarose gel stained with RedSafe in 1× TBE buffer at 5 V/cm for 1.5 h and sequenced by the Sanger method (Bioneer, South Korea).

was injected first and then immediately the corresponding adjuvant.

Measurements and observations

Colony counts were taken prior to treatment (pre-count) and at 3, 7, 14 and 21 days after treatment. Two most representative branches were chosen from each tree on each sampling date and the number of woolly aphid waxy colonies on a standard 10 cm branch length was counted. The Henderson–Tilton formula was used to calculate a percentage reduction in colony density:

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

where Ta = count in treated plot after treatment;

Tb = count in treated plot before treatment;

Ca = count in control plot after treatment;

Cb = count in control plot before treatment.

Statistical analysis

Data were analysed on an assessment date-by- assessment date basis. The effects of treatment, cultivar and treatment by cultivar interaction were evaluated using a two-way analysis of variance (ANOVA). The least significant difference (LSD) test was used to compare the means of treatments at the 5% probability level. All

statistical analysis were conducted with

GenStat software.

GenBank database of NCBI showed high similarity (590/591 bp = 99.83%, 0 gaps) with the sequence of *Eriosoma lanigerum* voucher CNC#HEM028323 (KR043025.1), which has a confirmed taxonomic identity. The identified organism's full taxonomic classification is: Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Paraneoptera; Hemiptera; Sternorrhyncha; Aphidomorpha; Aphidoidea; Pemphigidae; Eriosomatinae; Eriosomatini; Eriosoma. The isolate sequence from Iraq has been submitted to GenBank with accession number OP776810.1. The high sequence similarities have ensured that the species level was identified with no ambiguity, and that the identity of the target pest used throughout the study was validated.

RESULTS AND DISCUSSION

Molecular identification of the pest

The aphid species of the field collected were identified at the molecular level and were found to belong to the species *Eriosoma lanigerum* Hausmann. When COX1 universal primers were used in PCR amplification a single band approximately 720 bp in size was obtained (Figure 1B). The BLAST result of the obtained sequence (591 bp) against the

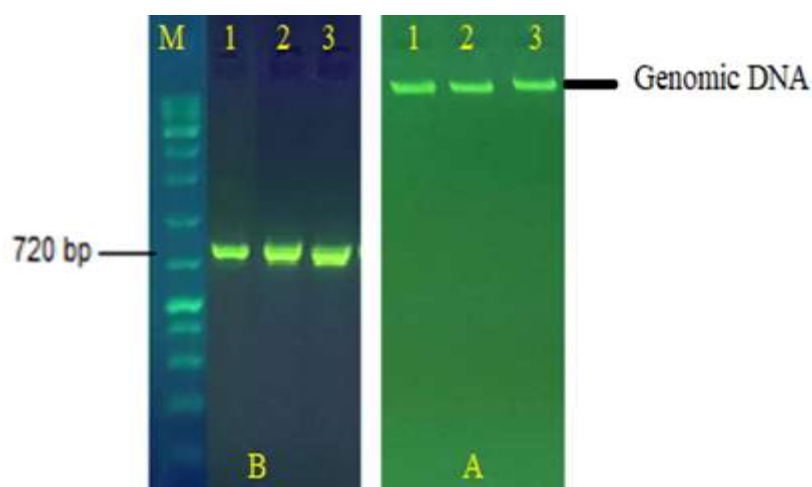


Figure 2. Molecular identification of the woolly apple aphid isolate based on the COX1 gene: (A) genomic DNA extraction gel electrophoresis from three insect species including *Eriosoma lanigerum* (lane 3), 1% agarose gel; (B) PCR amplification product at approximately 720 base pairs, 2% agarose gel; (C) BLAST alignment of the Iraqi isolate (Aw.J-3, OP776810.1) with *E. lanigerum* voucher CNC#HEM028323 (KR043025.1), showing 99.83% identity

Reduction percentage of woolly aphid waxy colonies

Sharabi cultivar

The reduction in woolly apple aphid waxy colonies varied significantly among the treatments at each measurement time on the cultivar Sharabi (Figure 3). The reduction percentage increased with time and ranged between 1.60–44.71% at 3 DPA to 51.92–94.26% at 21 DPA. The Closer + Bordeaux mixture treatment gave the highest reduction at all assessment dates

(44.71%, 63.06%, 77.42% and 94.26% at 3, 7, 14 and 21 days, respectively) and the overall mean reduction of 69.86% was significantly higher than all other treatments ($P < 0.05$). Next, Closer + potassium silicate treatment had an overall mean reduction of 63.94% whereas Closer alone treatment had an overall mean reduction of 54.23%.

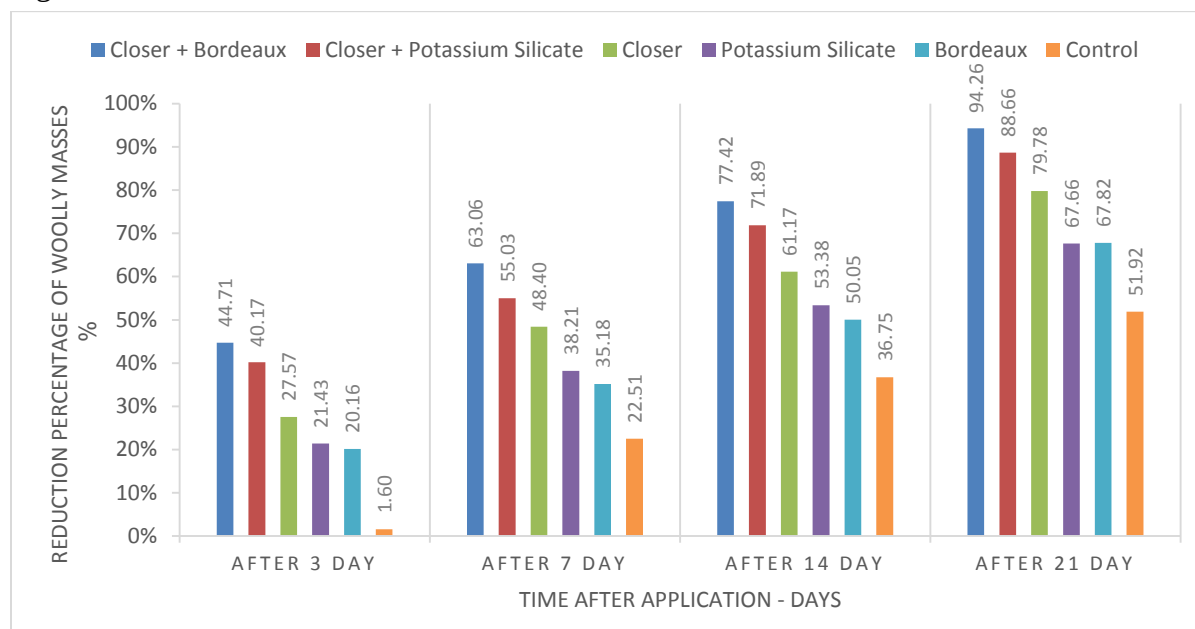


Figure (3). Woolly apple aphid (*Eriosoma lanigerum*): Percentage aphid waxy colonies removed by various treatments after various assessment periods on the Sharabi cultivar.

The better effectiveness of the Closer + Bordeaux mixture treatment could be due to the ability of Bordeaux mixture to fill bark crevices and protected microhabitats that are preferred by woolly aphids, making them less desirable feeding and reproductive sites and making the colonies more susceptible to the insecticide treatment. The results agree with those of [3], who found that accessibility to colonies in the crevices of bark is a critical component for effective woolly apple aphid control. The high efficacy of Closer reinforces the results of

[19] who found that sulfoxaflor is highly active against piercing-sucking insects and it affects nicotinic acetylcholine receptors. The extra protection provided by Closer + potassium silicate over the insecticide alone may be due to strengthening of plant surface tissues and activation of plant defenses as described by [8.9.17]

Ibrahimi cultivar

Ibrahimi cultivar showed a similar response to Sharabi (Figure 3) in general. The Closer + Bordeaux mixture treatment

was the most effective at all assessment dates with a mean reduction of 69.55% and the final reduction at 21 days of 92.76% ($P < 0.05$). The Closer + potassium silicate treatment was the second with a final

reduction of 87.58% and an overall mean value of 61.07%. The final reduction of closer alone was 78.62% with an overall mean of 53.05%.

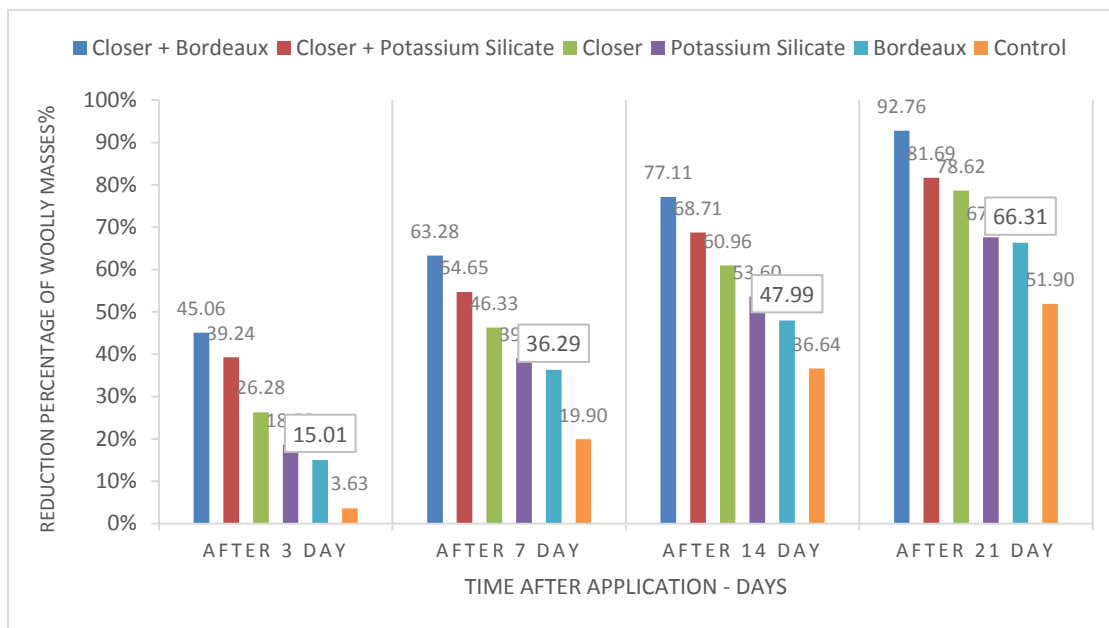


Figure (4). Woolly apple aphid (*Eriosoma lanigerum*): Percentage aphid waxy colonies removed by various treatments after various assessment periods on the Ibrahimi cultivar.

Treatment with Bordeaux mixture along with the insecticide showed a statistically significant higher reduction efficiency of 14.14% compared to the insecticide alone at 21 d. Likewise, the addition of potassium silicate to the insecticide produced a significant increase in the effectiveness of the pest control, which supports the hypothesis of a synergistic effect of silicon on the effectiveness of pest control through increased mechanical and physiological plant resistance. These results corroborate the findings of [13] who reported significant decrease in aphid colony density and feeding rate on silicon treated plants, and [12] who demonstrated that the

suitability of the host plants for piercing-sucking insects was reduced when treated with silicon. The findings are also consistent with [15] . [6] who reported reduction in aphid population with silicon application in a dose-dependent manner, and [18] who found that potassium silicate was effective against aphids under field conditions in Iraq. The effectiveness of sulfoxaflor-based products aligns with the findings of [14] and [20] and utilizing an adjuvant could also help to delay resistance build up in field populations.

Comparison of the most effective treatments between the two cultivars at 21 days post-application

The highest percentage reduction in waxy colonies was seen in the Closer + Bordeaux mixture treatment in both cultivars at 21 days post-application, and was 94.26% for Sharabi and 92.76% for Ibrahimi respectively (Figure 4). The cultivars of

Sharabi consistently produced a slightly higher response in all treatment groups, with the biggest inter-cultivar difference noted in the Closer + potassium silicate treatment (88.66% vs. 87.58%, a difference of about 1.08 percentage points at 21 days; a difference of 2.87 percentage points overall).

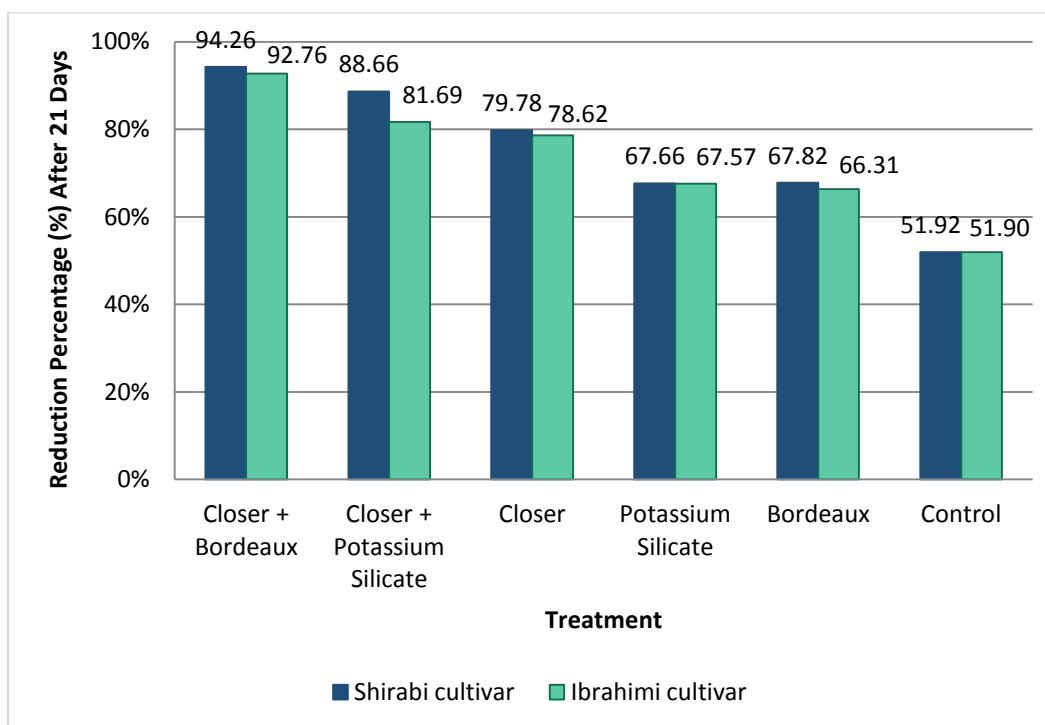


Figure (5). Comparison between Sharabi and Ibrahimi apple cultivars in the percentage reduction of woolly apple aphid waxy colonies after 21 days post-treatment

This inter-cultivar variation might be due to differences in the susceptibility of cultivars to infestation or differences in anatomical and biochemical resistance traits. In general, the data shows that Closer when applied

with Bordeaux mixture or potassium silicate leads to a significant increase in the control of woolly apple aphid when compared with the insecticide alone. The enhancing effect by Bordeaux mixture was higher in both cultivars.



Figure (6). Apple trees after application of the Closer + Bordeaux mixture treatment, showing a marked reduction in waxy colony density compared to the pre-treatment condition

CONCLUSION

The use of Closer (sulfoxaflor) along with either Bordeaux mixture or potassium silicate has been effective in controlling woolly apple aphid (*Eriosoma lanigerum*) to a large extent on both Sharabi and Ibrahimi apple cultivars as compared with the insecticide alone. The Closer + Bordeaux mixture treatment was the most effective followed by Closer + potassium silicate. Susceptibility differences among cultivars were observed with the Sharabi cultivar exhibiting a slightly higher response as compared to Ibrahimi. The Iraqi isolate

(Aw.J-3) had 99.83% similarity with the voucher CNC#HEM028323 of *E. lanigerum* when compared by sequence identity using COX1 gene and the sequence was registered in GenBank with accession number OP776810.1. The results corroborate that the use of Closer as a component of integrated pest management (IPM) programmes in apple orchards combined with Bordeaux mixture or potassium silicate is an effective and promising strategy, and can help to lower the use of chemical insecticides in pest control..

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