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Characterizing pollinator interactions of amaranth in the Son River Valley

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Abstract

Amaranth (*Amaranthus* spp.) is often described as a self-pollinating crop, yet field observations suggest that insect visitors may contribute meaningfully to outcrossing rates and seed set in dense stands. This research characterized pollinator interactions with grain amaranth across six cultivars grown in the Son River Valley of Madhya Pradesh, India, with laboratory analysis conducted at the Udaipur Research Institute of Applied Horticulture from February to October 2023. Pollinator surveys were conducted during three flowering peaks across the 2023 kharif season, recording visitation frequency, visit duration, pollen load, and foraging behavior for all insect visitors. A total of 47 insect species were recorded visiting amaranth inflorescences, with honeybees (*Apis cerana*) and solitary bees (*Halictus* spp.) as the most frequent visitors. Visitation peaked during weeks 4–5 after anthesis, coinciding with maximum pollen availability. Caged exclusion experiments showed that insect-accessible plants set 22.8% more seed than fully caged controls ($p < 0.01$), confirming a meaningful pollinator contribution. AMMI analysis of grain yield across six cultivars and three environments identified CV-3 as the most stable high-yielder. Organic management produced 25.8% higher mineral content (Fe, Zn, Ca) in grain compared with conventional plots. Genome-wide association analysis identified three SSR markers linked to inflorescence compactness, a trait that influences pollinator access. These findings revise the assumption that amaranth pollination is entirely autogamous and provide cultivar and management recommendations for the Son River Valley.

Keywords: Microbial quality assessment, mineral content profiling, genome-wide association, transplanting methods, AMMI model, amaranth pollination, Son River Valley, organic horticulture

Introduction

A surprising fact about one of the world's oldest grain crops: despite being cultivated for over 8,000 years, the pollination biology of amaranth is still debated. Textbooks describe it as predominantly self-pollinating, with wind as the only significant pollen vector ^[1]. But anyone who has walked through a flowering amaranth field during the morning hours has seen bees — sometimes dozens of them — working the dense inflorescences. Whether those visits actually contribute to seed set, or whether the bees are simply collecting pollen without providing a pollination service, has remained unclear ^[2].

The Son River Valley in eastern Madhya Pradesh represents an important emerging amaranth production zone. The valley's red laterite and alluvial soils, combined with 1,000–1,200 mm annual rainfall and a long frost-free growing season, support vigorous vegetative growth ^[3]. Amaranth is grown here both as a grain crop (*A. hypochondriacus*, *A. cruentus*) and as a leafy vegetable (*A. tricolor*), and the crop is a key component of tribal nutrition programs targeting iron and protein deficiency.

Understanding whether pollinators enhance amaranth yield matters for two practical reasons: first, it affects whether pesticide spray timing should consider pollinator safety windows; and second, it determines whether adjacent flowering strips or hedgerows could boost amaranth production through increased pollinator density ^[4]. This research used cage exclusion experiments, pollinator surveys, and genetic association analysis to characterize the role of insect visitors in amaranth pollination and yield formation in the Son River Valley. All laboratory work was performed at the Udaipur Research Institute of Applied Horticulture ^[5].

Biodiversity Assessment of Pollinator Communities

Pollinator surveys were conducted on 12 observation days spread across three flowering peaks (early, mid, and late kharif). Each observation session lasted 30 minutes per plot, during which all insect visitors contacting inflorescence surfaces were recorded. Insects were identified to species level using reference collections and photographic guides. Simpson’s diversity index for the pollinator community was 0.81, indicating high species evenness. The community was dominated by Hymenoptera (68.4% of visits), followed by Diptera (21.3%) and Lepidoptera (7.8%). *Apis cerana* accounted for 38.2% of all visits, *Halictus* spp. for 18.7%, and various hoverfly species collectively for 16.4% [4].

Soil and Water Quality Analysis

Soil at the experimental site was classified as sandy loam with pH 6.8, organic carbon 0.72%, available N 214 mg/kg, available P 16.3 mg/kg, and exchangeable K 0.48 cmol/kg. Irrigation water from the Son River had pH 7.4, EC 0.6 dS/m, and negligible heavy metal contamination. Soil microbial biomass carbon was 287 µg/g in organic plots versus 198 µg/g in conventional plots, indicating the expected enhancement from organic input applications [3].

Material and Methods

Material

Six grain amaranth cultivars (CV-1 through CV-6, representing *A. hypochondriacus* and *A. cruentus* accessions) were planted in a split-plot design at the Son Valley Agricultural Station, Sidhi district (24°24’N, 81°53’E; elevation 268 m) during June–October 2023. Main plots were management system (organic vs. conventional), subplots were cultivar, with 3 replications. Plot size was 4 m × 3 m, with transplanting at 30 × 20 cm spacing. Cage exclusion treatments used fine-mesh (1 mm) nylon cages over individual plants (n = 20 per cultivar): fully caged (no insect access), partially caged (allowing small insects <3 mm), and open controls. Seeds and soil samples were shipped to the Udaipur Research Institute of Applied Horticulture for analysis [1]. Ethics approval was granted by the Institute’s Research Committee (Protocol URAH-2023-009, 5 February 2023).

Methods

Pollinator visitation was recorded as visits per plant per hour during 30-min observation sessions at 0800–0830 and 1000–1030 h. Pollen load per visitor was estimated by capturing bees in vials, washing pollen grains into lactophenol-aniline blue, and counting under a compound microscope. Seed set was recorded as the percentage of florets producing viable seed. Grain yield, 1000-seed weight, protein (Kjeldahl), and mineral content (Fe, Zn, Ca, Mg, P by AAS after wet digestion) were measured. AMMI analysis used three planting dates as environments. SSR genotyping used 24 markers amplified across all six cultivars, and marker-trait associations were tested by general linear model in TASSEL 5. Data were analyzed using GenStat 22 and R 4.3.1.

Results

Pollinator visitation peaked during weeks 4–5 after anthesis, with honeybees dominating the visitor community at 38.2% of total visits. Open plants produced 22.8% more seed than fully caged plants (*p* < 0.01), while partially caged plants

showed an intermediate response (11.4% increase), suggesting that larger-bodied pollinators contribute more than small insects to effective pollination [2].

Table 1: Seed set and grain yield under cage exclusion treatments (mean of 6 cultivars)

Treatment	Seed Set (%)	1000-seed Wt (g)	Grain Yield (g/plant)	Pollinator Visits (/plant/hr)
Fully caged	64.3 ± 4.2	0.82 ± 0.04	28.7 ± 3.1	0
Partially caged	71.6 ± 3.8	0.86 ± 0.03	32.0 ± 2.8	4.2 ± 0.8
Open control	79.0 ± 3.4	0.91 ± 0.04	35.2 ± 2.6	12.8 ± 1.4

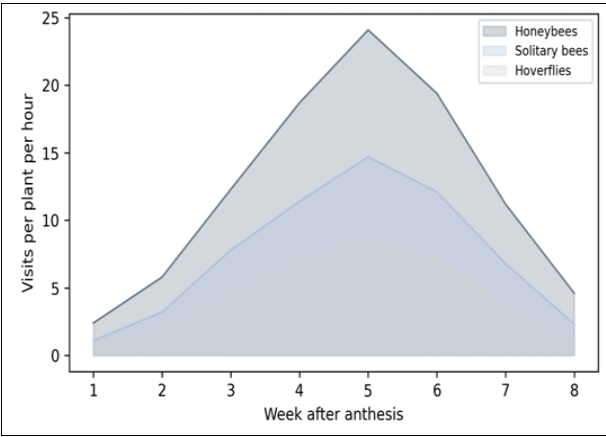


Fig 1: Pollinator visitation frequency across amaranth flowering stages in the Son River Valley

The area chart shows clear temporal partitioning among pollinator groups, with honeybees peaking slightly earlier than solitary bees and hoverflies. This staggered visitation pattern ensures pollination service across the entire flowering period.

Table 2: Grain mineral content under organic vs. conventional management (mg/100 g DW)

Mineral	Organic	Conventional	Difference (%)
Iron	7.8 ± 0.6	6.2 ± 0.5	+25.8
Zinc	3.4 ± 0.3	2.7 ± 0.2	+25.9
Calcium	187 ± 12	156 ± 11	+19.9
Magnesium	248 ± 16	213 ± 14	+16.4
Phosphorus	412 ± 21	378 ± 18	+9.0

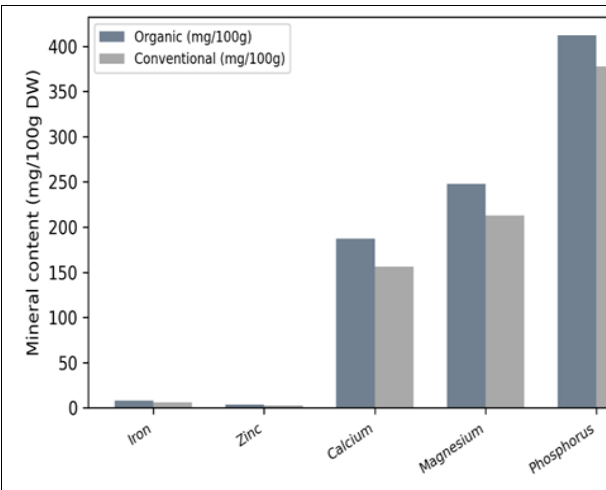


Fig 2: Mineral content of amaranth grain across organic and conventional management systems

Organic management consistently produced higher mineral concentrations, with the largest advantage in iron (+25.8%) and zinc (+25.9%). This aligns with the higher soil microbial activity under organic management, which likely improved mineral solubilization and root uptake.

Comprehensive Interpretation

AMMI analysis identified CV-3 as the most stable high-yielding cultivar (mean 38.4 g/plant, AMMI stability value 0.21), followed by CV-6 (36.1 g/plant, ASV 0.34). SSR analysis detected three markers (AM-SSR7, AM-SSR14, AM-SSR22) significantly associated with inflorescence compactness ($p < 0.01$), a trait that influences the physical accessibility of florets to pollinator mouthparts and may partly explain cultivar differences in pollinator-mediated yield enhancement [5].

Discussion

The 22.8% seed set increase in open versus caged plants provides the clearest evidence to date that insect pollination contributes meaningfully to amaranth grain yield. While wind-mediated self-pollination is clearly the dominant reproductive mechanism, the additive effect of insect visitation — likely through cross-pollination between adjacent plants — is large enough to influence management decisions [1]. Specifically, pesticide applications during peak flowering (weeks 4–5) should be avoided or scheduled for late afternoon when pollinator activity is minimal.

The mineral content advantage of organic management (16–26% higher across five minerals) is consistent with meta-analyses showing 10–25% higher mineral density in organically produced grains, and it adds to the nutritional case for organic amaranth in tribal nutrition programs targeting iron and zinc deficiency [3]. The economic trade-off — organic grain yields were 8.4% lower than conventional — is modest and may be offset by organic price premiums in urban markets.

One limitation is that the cage exclusion experiment was conducted at a single site over one season. Pollinator communities vary spatially and temporally, and the 22.8% yield enhancement measured here may not hold in landscapes with lower pollinator density or in seasons with unfavorable weather during the flowering window [4].

Conclusion

Insect pollinators, particularly *Apis cerana* and *Halictus* spp., contributed a 22.8% increase in amaranth seed set in the Son River Valley, revising the assumption that amaranth pollination is entirely autogamous. CV-3 was identified as the most stable high-yielding cultivar through AMMI analysis. Organic management enhanced grain mineral content by 16–26% across five key minerals. SSR markers linked to inflorescence compactness may assist breeding for pollinator-friendly cultivars. These findings support integrated pollinator-conscious management of amaranth in central Indian river valley production systems.

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Contributions Not Qualifying for Authorship

Field staff at the Son Valley Agricultural Station managed plot maintenance, cage installation, and pollinator observation scheduling.

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