



Eco-friendly control of mosquito vectors using *Cajanus Cajan* peel extracts

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Abstract

Cajanus cajan, commonly known as pigeon pea, is a widely cultivated monocotyledonous perennial plant belonging to the Fabaceae family. In India, it is primarily grown for its seeds, known as toor dal, which are extensively used in culinary and industrial applications. The processing of *C. cajan* generates substantial amounts of peel residues, which, if unutilized, contribute significantly to environmental pollution. In this study, an eco-friendly approach was employed to valorize these agricultural waste peels by extracting their bioactive metabolites using four different solvents: aqueous, chloroform, hexane, and methanol. The larvicidal efficacy of the solvent extracts was evaluated against fourth instar larvae of three major mosquito vectors—*Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*.

All extracts exhibited varying degrees of larvicidal activity, with methanol and chloroform extracts showing the highest potency. Phytochemical screening, Thin Layer Chromatography (TLC), and Gas Chromatography-Mass Spectrometry (GC-MS) analyses confirmed the presence of bioactive compounds such as flavonoids, tannins, terpenoids, and carbohydrates, which are likely responsible for the observed mosquitocidal effects. The findings suggest that *Cajanus cajan* peel extracts, particularly from methanol and chloroform fractions, have significant potential as natural larvicides. This study supports the development of sustainable, plant-based mosquito control strategies and highlights the potential of agro-waste utilization in vector management programs. Future work will focus on isolating and characterizing the specific phytochemicals responsible for larvicidal activity and elucidating their mechanisms of action.

Keywords: *Cajanus cajan*, mosquito larvicidal activity, phytochemical analysis, agro-waste utilization, vector control

Introduction

Mosquito-borne diseases continue to impose a heavy toll on global health, particularly in tropical and subtropical regions. According to Muhammad *et al.* (2017) [38], these illnesses are endemic in over 100 countries and are responsible for nearly two million deaths annually. Tragically, around one million of those deaths occur in children, and an estimated 2.1 billion people remain at risk worldwide. The pervasive nature of mosquito vectors and the diseases they transmit—such as malaria, dengue, chikungunya, yellow fever, lymphatic filariasis, and viral encephalitis—are major contributors to global morbidity and mortality, particularly in developing countries with limited healthcare resources (Anoopkumar & Aneesh, 2022) [6].

India, with its diverse and climatically favorable ecological zones, faces a particularly acute burden from mosquito-borne diseases. Sumodan Elumalai *et al.* (2016) [53] highlight that the country's tropical and subtropical climate, coupled with rapid urbanization and insufficient sanitation, creates an environment where mosquito populations thrive. Joshi (2018) [23] reports that 17 Indian states and six Union Territories are endemic to mosquito-borne diseases, placing roughly 553 million individuals at risk of infection. Such high exposure underscores the critical need for robust epidemiological surveillance, vector control strategies, and public health infrastructure.

Among the vector species, *Anopheles stephensi* plays a pivotal role in malaria transmission within both urban and rural landscapes of India and other regions (Subbarao *et al.*, 2019) [51]. This species thrives in small, artificial water-holding containers, commonly found at construction sites,

leading to recurrent urban malaria outbreaks (Veni *et al.*, 2017). Globally, the burden remains significant: in 2005, approximately 2.5 billion people were considered at risk of malaria, with about 2.7 million deaths annually (Nnamonu *et al.*, 2020) [40]. The persistence of such figures emphasizes the ongoing necessity for efficient vector control measures.

Another prominent mosquito vector in India is *Aedes aegypti*, which is responsible for transmitting dengue fever, dengue hemorrhagic fever, chikungunya fever, and yellow fever. This species is prevalent across tropical and subtropical regions, contributing significantly to arboviral disease outbreaks (Otu *et al.*, 2019) [41]. The World Health Organization (2009) estimates that approximately two-fifths of the global population is at risk of dengue. In India, the incidence of dengue reached 28,292 cases with 110 fatalities by 2010 (Patali *et al.*, 2018) [42]. The co-circulation of multiple dengue serotypes and other viruses carried by *Aedes* spp. complicates disease dynamics and presents serious public health concerns.

The mosquito species *Culex quinquefasciatus* is the primary vector of lymphatic filariasis, a disease that afflicts nearly 120 million people globally, with approximately 44 million suffering from chronic manifestations such as lymphedema and elephantiasis (Gordon *et al.*, 2018) [21]. Even though lymphatic filariasis can cause severe disability, it often receives low priority in public health initiatives (Pisarski, 2019) [43]. Endemic regions, including India, struggle with persistent transmission despite long-standing mass drug administration programs, underscoring the need for integrated vector management strategies.

For decades, synthetic chemical insecticides have been the cornerstone of mosquito control programs. However, their

sustained use has led to several serious drawbacks. Chief among these is the evolution of insecticide resistance in mosquito populations, which diminishes the efficacy of control efforts (Dahmana *et al.*, 2020) [14]. Synthetic insecticides also affect non-target organisms and disrupt ecological balances, posing significant risks to biodiversity (Khan & Ahmad, 2019) [25]. Many of these chemicals persist in the environment, accumulate in water and soil, and pose potential toxic risks to humans and animals (Sharma *et al.*, 2020) [48].

Moreover, the health repercussions of direct human exposure to chemicals in mosquito repellent products cannot be overlooked. Popular items like mosquito coils and sprays contain synthetic pyrethroids and organophosphates, substances linked to respiratory issues, eye and skin irritation, headaches, asthma exacerbation, and allergic reactions (Mandal, 2019) [34]. The indoor residual spraying of insecticides, traditionally used to target malaria vectors, often leaves a lingering odor and unsightly stains on walls, which reduces public acceptance (Suuron *et al.*, 2020) [54]. Additionally, electric mosquito repellents and vaporizers—common in households, eateries, and healthcare facilities—contain volatile compounds that may exert long-term health effects (Eliopoulos *et al.*, 2018) [16].

The cumulative drawbacks of synthetic insecticides—resistance development, non-target impacts, ecological disruption, and adverse human health outcomes—have led researchers and public health practitioners to reconsider reliance on chemical interventions (Dahmana & Mediannikov, 2020) [14]. This has catalyzed a growing interest in alternative, eco-friendly vector control options.

Plants represent an abundant source of biologically active compounds that can serve as natural insecticides, repellents, ovicides, or larvicides. Numerous studies have explored plant-origin natural products for mosquito control, demonstrating their biodegradability, lower toxicity to non-target species, and affordability (CE *et al.*, 2019). Many botanical compounds, including essential oils, alkaloids, terpenoids, and phenolics, have shown promising mosquito-control properties. These substances often exhibit multiple mechanisms of action—such as neurotoxicity, growth inhibition, or behavioral disruption—making it difficult for insects to develop resistance.

For instance, essential oils derived from *Lemongrass* (*Cymbopogon citratus*), *Neem* (*Azadirachta indica*), and *Eucalyptus* have been extensively tested for their repellent and larvicidal effects and have shown encouraging results in field trials and laboratory studies. Botanical agrochemicals can also be formulated as emulsifiable concentrates, microencapsulations, and biodegradable gels to suit diverse application methods ranging from indoor surface sprays to ovitraps and spatial repellents. Plant-based vector control fits well within the framework of integrated pest management (IPM). Combining botanical agents with environmental management practices—such as eliminating breeding sites, improving water and waste sanitation, and public awareness campaigns—can substantially reduce mosquito populations while minimizing chemical exposure.

The global burden of mosquito-borne diseases persists despite decades of vector control efforts. The reliance on synthetic insecticides has resulted in resistance, ecological damage, and health risks. In India, where mosquito-borne illnesses such as malaria, dengue, lymphatic filariasis, and

viral encephalitis are highly prevalent due to favorable environmental and societal conditions, the need for effective control strategies is particularly urgent. Natural, plant-based mosquito control agents offer a promising, sustainable, and eco-friendly alternative that aligns with global health and environmental objectives. Scaling up research and application of botanical-based interventions could greatly mitigate the public health burden and advance responsible vector management practices.

Cajanus cajan (L.) Millsp., commonly known as pigeon pea, is a leguminous crop widely grown in tropical and subtropical regions, with extensive applications in traditional medicine. Phytochemical analyses of various parts of the plant—particularly the roots, leaves, and seeds—have revealed a rich diversity of bioactive compounds. These include isoflavonoids such as genistein, cajanol, formononetin, and daidzein; flavonoids like quercetin, orientin, and vitexin; stilbenes including cajaninstilbene acid (CSA) and longistylins A and C; as well as anthocyanins (e.g., cyanidin-3-monoglucoside), coumarins, phenolic acids, and terpenoids (Popović *et al.*, 2011; Lai *et al.*, 2012; Kunnumakkara *et al.*, 2016) [44, 28, 26].

The distribution of these compounds varies across plant parts, with leaves and roots being particularly rich in flavonoids and stilbenes, while seeds contain notable levels of isoflavones and anthraquinones. Biological studies have demonstrated a broad spectrum of pharmacological effects. Antioxidant properties are among the most well-characterized, with ethanol and aqueous extracts of leaves showing significant radical-scavenging activity via DPPH and β -carotene bleaching assays, primarily attributed to CSA, pinostrobin, and vitexin (Lai *et al.*, 2012; Ersam *et al.*, 2018) [28, 17]. Furthermore, the plant exhibits antimicrobial properties: acetone and methanolic seed and leaf extracts are effective against *Staphylococcus aureus*, *E. coli*, and *Candida albicans*, often at low MIC values, due to compounds such as formononetin, biochanin A, and rhein (Balogun *et al.*, 2022) [19].

Overall, the phytochemical richness of *C. cajan*, particularly its abundance of isoflavonoids, flavones, and stilbenes, accounts for its wide range of therapeutic properties, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, anticancer, hypolipidemic, and anthelmintic effects, thus validating its traditional use and highlighting its potential for pharmacological and nutraceutical development.

In the present study, we aimed to evaluate the efficacy of *Cajanus cajan* peel extracts against the mosquito vectors *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. Additionally, the extracts were analyzed for the presence of secondary metabolites

Materials and Methods

Plant Material Collection

Healthy and disease-free peels of *Cajanus cajan* (L.), commonly known as pigeon pea and belonging to the family Fabaceae, were carefully collected from Adhanur village, located in the Villupuram district, Tamil Nadu, India. The selected peels were sourced from mature, freshly harvested fruits and were free from visible signs of pest or fungal infestation to ensure the quality and consistency of the experimental samples.



Figure 1: *Cajanus cajan* (L.)

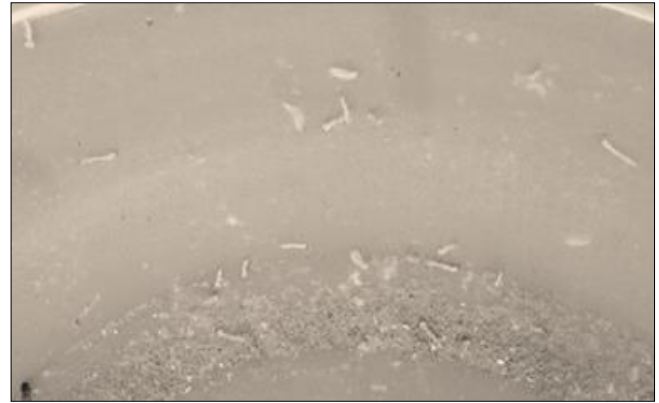


Figure 2: Selection of mosquito species

Selection of mosquito species

In the present study, fourth instar larvae of three mosquito species—*Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*—were selected due to their significant role in the transmission of arboviral and parasitic diseases. *Aedes aegypti*, a highly domesticated and anthropophilic species, is the primary vector for arboviruses such as dengue and dengue hemorrhagic fever (Foster & Walker, 2019) [19]. *Anopheles stephensi* is a recognized urban malaria vector known for transmitting *Plasmodium falciparum*, the most virulent malaria-causing parasite (Saif, 2017), while *C. quinquefasciatus* is responsible for spreading *Wuchereria bancrofti*, the causative agent of lymphatic filariasis, and is widely distributed in tropical regions (Nchoutpouen *et al.*, 2019) [39]. Larvae were reared under controlled insectary conditions ($26 \pm 2^\circ\text{C}$, $75 \pm 5\%$ RH, 12:12 h light–dark cycle) and fed a standard diet of yeast and dog biscuit (3:1). Healthy fourth instar larvae were exposed to various concentrations of *Cajanus cajan* peel extract in 100 mL dechlorinated water, with 25 larvae per concentration and three replicates per treatment. Mortality was recorded after 24 and 48 hours, and results were corrected using Abbott's formula where necessary. Lethal concentration values (LC_{50} and LC_{90}) were calculated through probit analysis using statistical software. The bioassays were conducted following WHO (2005) guidelines with appropriate negative and positive controls.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Statistical Analysis

Larval mortality data were analyzed using probit analysis to calculate LC_{50} and LC_{90} values with 95% confidence intervals, following Finney (1971) [18] and Busvine (1971). The goodness of fit was assessed by chi-square (χ^2) tests. All analyses were performed using software by Han *et al.* (2013) and SPSS v11.5. Results with $p < 0.05$ were considered statistically significant.

Results

Qualitative Phytochemical Analysis of *Cajanus cajan*

The qualitative phytochemical screening of *Cajanus cajan* peel extracts revealed a diverse array of bioactive compounds, varying in intensity across different solvents. The methanolic extract exhibited a strong presence of key secondary metabolites, including alkaloids, carbohydrates, saponins, glycosides, terpenoids, triterpenoids, and phenols, indicating the high polarity of methanol as an efficient

Phytochemical Screening

Preliminary phytochemical screening of the methanolic extract of *Cajanus cajan* peels was carried out according to standard protocols as described by Nweze *et al.* (2004) and Senthil Kumar and Reetha (2009). The extract was qualitatively tested for the presence of the following secondary metabolites: carbohydrates, alkaloids, flavonoids, phytosterols and steroids, anthocyanins and betacyanins, phenols, tannins, saponins, glycosides, and proteins. Standard colorimetric and precipitation-based assays were used for detection, and results were recorded visually based on characteristic color changes or precipitate formation.

Thin Layer Chromatography (TLC) Analysis

TLC was performed for profiling the methanol extract. Silica gel 60 F₂₅₄ aluminum-backed plates were cut to dimensions of 1.5 × 5.5 cm. A small volume of the methanolic peel extract was spotted onto the plates using a fine capillary tube, and the plates were allowed to air dry. The chromatographic separation was achieved using a solvent system consisting of hexane: ethyl acetate: chloroform in a 2:1:1 ratio as the mobile phase, following the protocol modified from Luong *et al.* (2021). The developed TLC plates were visualized under UV light at 254 nm and 366 nm, and the R_f values of separated bands were recorded for analysis.

solvent for phytochemical extraction. Moderate levels of tannins, cardiac glycosides, and steroids were also detected in the methanol extract, suggesting the presence of additional phytoconstituents that may contribute to biological activity, albeit in lesser concentrations. However, coumarins, quinones, anthocyanins, and flavonoids were found to be absent in the methanol extract, indicating that either these compounds are not present in *C. cajan* peels or are present in concentrations below the detection limits of the employed qualitative assays.

In comparison, the aqueous, chloroform, and hexane extracts also showed the moderate presence of various phytochemicals, though with less intensity than the methanol extract. This variation underscores the solvent-dependent extraction efficiency, as non-polar solvents like hexane tend to be less effective in extracting polar phytochemicals such as glycosides and phenolic

compounds. The aqueous extract retained moderate levels of polar compounds, while chloroform revealed moderate quantities of both polar and non-polar phytochemicals, consistent with its intermediate polarity. Overall, the methanol extract demonstrated the richest phytochemical profile, suggesting that it is the most suitable solvent for isolating a broad spectrum of bioactive compounds from *Cajanus cajan* peels. These findings are further supported by Table 1, which presents a comparative overview of the phytochemical constituents across all four solvent extracts.

Larvicidal Activity of *Cajanus cajan* Peel Extracts

The larvicidal potential of *Cajanus cajan* peel extracts was evaluated against fourth instar larvae of three vector mosquito species: *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. The mortality data were subjected to probit analysis, and the lethal concentration values (LC₅₀ and LC₉₀) after 24 hours of exposure. Among the solvent extracts tested, the methanol extract demonstrated the highest larvicidal efficacy, exhibiting strong toxicity against all three-mosquito species. The extract was most potent against *C. quinquefasciatus*, with an LC₅₀ value of 36.089 ppm and an LC₉₀ value of 61.133 ppm, followed by *A. stephensi*, which exhibited an LC₅₀ of 47.892 ppm and an LC₉₀ of 95.716 ppm. *A. aegypti* was comparatively less susceptible, with LC₅₀ and LC₉₀ values of 50.500 ppm and 88.340 ppm, respectively. These findings suggest that *C. cajan* peel methanol extract is particularly effective against filarial and malarial vectors (*Culex* and *Anopheles* spp.) at lower concentrations.

The aqueous extract of *C. cajan* also exhibited larvicidal activity, though with slightly higher LC values. For *A. stephensi*, the aqueous extract yielded an LC₅₀ of 52.537 ppm and an LC₉₀ of 117.639 ppm, while *C. quinquefasciatus* showed LC₅₀ and LC₉₀ values of 56.163 ppm and 122.667 ppm, respectively. *A. aegypti* again appeared more resistant, with LC₅₀ and LC₉₀ values of 63.327 ppm and 128.635 ppm, respectively. In contrast, the chloroform and hexane extracts demonstrated larvicidal effects only at higher concentrations, indicating lower efficacy compared to methanol and aqueous extracts. This outcome reflects the relatively lower polarity of hexane and chloroform, which likely results in limited extraction of key bioactive constituents responsible for larvicidal activity.

The results clearly indicate that the methanolic peel extract of *Cajanus cajan* contains potent phytochemicals capable of inducing significant larval mortality, particularly in *C. quinquefasciatus* and *A. stephensi*. Owing to its superior performance, the methanol extract was selected for further phytochemical profiling using TLC and GC-MS techniques to identify the active constituents responsible for the observed bioactivity.

These findings support the potential of *Cajanus cajan* peel, a commonly discarded agro-waste, as a valuable source of eco-friendly larvicidal agents for vector control strategies.

Thin Layer Chromatography (Tlc) Analysis

Thin Layer Chromatography (TLC) analysis of the methanolic peel extract of *Cajanus cajan* revealed the presence of eleven distinct bands with retention factor (Rf) values of 0.75, 0.71, 0.60, 0.52, 0.48, 0.46, 0.37, 0.32, 0.29, 0.24, and 0.19. These bands correspond to a variety of phytochemical compounds, including triterpenoids, steroids, phenolic compounds, glycosides, anthocyanins, saponins,

triterpenes, alcohols, terpenoids, phenols, and alkaloids, respectively. The diversity and number of separated bands indicate that methanol effectively extracts a broad spectrum of bioactive constituents from *Cajanus cajan* peels. The presence of bioactive classes such as alkaloids, phenolics, and saponins aligns with the plant's demonstrated larvicidal and medicinal properties. The well-resolved bands further suggest that the chosen solvent system (hexane: ethyl acetate: chloroform in a 2:1:1 ratio) provided efficient separation of phytochemicals, confirming the rich phytochemical profile of *Cajanus cajan* peels. This complex mixture of secondary metabolites highlights the potential of *Cajanus cajan* peel extracts for use in natural product development, particularly for vector control applications.

Discussion

Numerous studies have documented the efficacy of phytochemicals derived from various plant species in mosquito control, demonstrating their potential as alternative, eco-friendly insecticides (Ali *et al.*, 2013) [4]. Sukumar *et al.* extensively reviewed botanical derivatives tested against mosquitoes and highlighted many plant extracts with larvicidal or repellent properties; however, they noted that only a few have shown practical value for large-scale mosquito control applications (Malathi and Vasugi, 2015; Bekele, 2018) [33, 11]. Plants are rich reservoirs of complex bioactive compounds that can serve as environmentally safe agents for vector and pest management (Tennyson *et al.*, 2018) [55]. Natural pesticides derived from plants remain particularly promising tools for targeting mosquito larvae, offering an effective strategy to reduce vector populations while minimizing environmental hazards (Lebon *et al.*, 2018) [29].

In the present study, methanol, hexane, chloroform, and aqueous peel extracts of *Cajanus cajan* demonstrated significant larvicidal activity against the fourth instar larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. This research marks the first report investigating the larvicidal efficacy of *Cajanus cajan* peel extracts. The results align with previous observations by Rodrigues (2020) [46], supporting the potential of fruit peels as bioinsecticides. Comparable larvicidal effects were observed by Marin *et al.* (2020) [36], who reported the ethanolic peel extract of *Citrus sinensis* to be more toxic against *A. aegypti* (LC₅₀ = 92.27 ppm) than *C. quinquefasciatus* (LC₅₀ = 244.70 ppm) (Aggarwal *et al.*, 2020) [2]. Our study further revealed potent larvicidal activity of methanol peel extracts of *Cajanus cajan*, with the highest toxicity recorded against *C. quinquefasciatus* (LC₅₀ = 36.089 ppm; LC₉₀ = 61.133 ppm) and *A. stephensi* (LC₅₀ = 47.892 ppm; LC₉₀ = 95.716 ppm). These findings correspond with the repellency and larvicidal behaviors reported in related studies, such as those by Kamaraj *et al.* (2023) [24], who observed significant mosquito repellency using *Parthenium hysterophorus* leaf extracts. Such consistent results highlight the potential of plant-based extracts as alternative mosquito control agents.

The phytochemicals found in *Cajanus cajan*—including terpenoids, flavonoids, saponins, tannins, steroids, and alkaloids—are known to exhibit diverse biological activities such as larvicidal, insect growth regulatory, repellent, and oviposition deterrent effects (Srinivasan *et al.*, 2014; Muema *et al.*, 2017) [50, 37]. Triterpenoids, in particular, have been widely credited with mosquito larvicidal properties

(Manikandan *et al.*, 2022) [35]. Studies on other plants like *Ocimum sanctum* have also linked larvicidal efficacy to the presence of alkaloids, flavonoids, and tannins (Anees *et al.*, 2008; Azis Ikhsanudin and Ramadani, 2021) [5, 8]. Moreover, essential oils and extracts from species such as *Cassia fistula* and *Clerodendrum bonplandianum* have been reported to exert strong larvicidal and repellent effects against mosquito larvae and adults, further supporting the role of phytochemicals in vector control (Athira *et al.*, 2016; Ghosh *et al.*, 2018) [7, 20]. The presence of similar phytochemical groups in *Cajanus cajan* peel extracts strengthens the case for their potential use as natural insecticides (Sowjanya *et al.*, 2013; Ahmed *et al.*, 2019; Bisht *et al.*, 2021) [49, 3, 12].

The strong larvicidal activity observed in *Cajanus cajan* peel extracts may be attributed to the synergistic effects of terpenoids and triterpenoids, which are known to disrupt insect development and physiology (Velu *et al.*, 2015; Baseer and Jain, 2016; Saravanan, 2022; Dhama *et al.*, 2023) [57, 10, 47, 15]. Qualitative phytochemical analysis showed the methanol peel extracts to be rich in tannins, saponins, phenols, quinones, cardiac glycosides, alkaloids, and terpenoids—compounds previously confirmed to possess insecticidal properties by TLC and GC-MS analyses. The variability in larvicidal potency among different extracts and mosquito species may be influenced by factors such as the polarity of extraction solvents and the specific bioactive compound profiles they yield (Veni *et al.*, 2017; Raveen *et al.*, 2017) [45]. Methanol extracts, in particular, demonstrated superior efficacy at relatively low concentrations compared to other solvents, highlighting their potential for further development as larvicidal agents. In summary, the peel extracts of *Cajanus cajan* show significant promise as potent, eco-friendly larvicides against important mosquito vectors. These findings pave the way for future investigations into the isolation, characterization, and formulation of active compounds for sustainable mosquito management strategies.

Conclusion

Our studies clearly demonstrate that waste peels of *Cajanus cajan* can serve as effective agents for mosquito control. Utilizing these agricultural wastes not only helps reduce environmental burden by managing otherwise intractable waste but also decreases pollution and enhances the ecological sustainability of the fruit processing industry.

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