Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Cytotoxic, Phytotoxic and Insecticidal Potential of *Achillea millefolium* L. and *Chaerophyllum villosum* wall. ex dc.

Potencial citotóxico, fitotóxico e insecticida de *Achillea Millefolium* L. e *Chaerophyllum Villosum*

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Abstract

The methanolic, chloroformic and aqueous extract of *Achillea millefolium* and *Chaerophyllum villosum* were investigated for cytotoxicity, phytotoxic and insecticidal activities. Cytotoxicity was investigated by brine shrimp lethality assay indicating that the crude methanolic extract of *A.millefolium* and chloroformic extract of *C.villosum* revealed highest mortality of brine shrimps with (LD_{50} of 52.60 µg/ml) and (14.81 µg/ml). Phytotoxicity was evaluated using the *Lemna minor* bioassay which revealed that the crude methanolic extract of *A.millefolium* and *C.villosum* extract has maximum inhibition of *Lemna minor* with (Fl_{50} 6.60 µg/ml) and (0.67 µg/ml).The insecticidal activity showed that among all the insects studied it was observed that methanolic extract of *A. millefolium*and *C. villosum* was highly toxic to *Sphenoptera dadkhani* with (LD_{50}=4.17 µg/ml) and (0.34 µg/ml). From the present study it can be concluded that different extracts from *A. millefolium* and *C. villosum* showed good cytotoxic, phytotoxic and insecticidal activity in a dose dependent manner.

Keywords: cytotoxic, phytotoxic, insecticidal activity, *Achillea millefolium, Chaerophyllum villosum.*

Resumo

Neste estudo, os extratos metanólico, clorofórmico e aquoso de *Achillea millefolium* e *Chaerophyllum villosum* foram analisados em relação à citotoxicidade, atividade fitotóxica e inseticida. A citotoxicidade foi analisada através do ensaio de letalidade de artémia, indicando que o extrato metanólico bruto de *A. millefolium* e o extrato clorofórmico de *C. villosum* revelaram maior mortalidade de artêmias com DL50 de 52,60 µg/ml e 14,81 µg/ml. A fitotoxicidade foi avaliada utilizando o bioensaio de *Lemna minor* que revelou que o extrato metanólico bruto de *A. millefolium* e extrato de *C. villosum* têm inibição máxima de Lemna minor com Fl50 6,60 µg/ml e 0,67 µg/ml. A atividade inseticida mostrou que dentre todos os insetos estudados, o extrato metanólico de *A. millefolium* e de *C. villosum* foi altamente tóxico para *Sphenoptera dadkhani*com DL50 = 4,17 µg/ml e 0,34 µg/ml . Por outro lado, diferentes extratos, como *A. millefolium* e C. villosum apresentaram boa atividade citotóxica, fitotóxica e inseticida de forma dose-dependente.

Palavras-chave: citotóxico, fitotóxico, atividade inseticida, *Achillea millefolium*, *Chaerophyllum villosum*.

1. Introduction

Traditional medicines are extensively used from many years due to their security. In many developing countries a large number of the population uses medicinal plants for treatment of several ailments (Vendruscolo et al., 2022; Nguta et al., 2011). Plants contain certain active constituents which are important medicinally. These phytochemical constituents are present in storage organs of the botanicals (Himesh et al., 2011). Different varieties of compounds extracted from plant parts are used as protective agents against viral, fungal, bacterial, and insecticidal diseases in plants. They may also act as scavengers of free radicals, absorbing UV lights, act against proliferative stimuli along with acting as being antioxidant in nature (Lillo et al., 2023).

In order to detect the antitumor compounds and the toxicity of plants towards cancer cells brine shrimp lethality bioassay is used (Olowa and Nuñeza, 2013). Phytochemicals are synthesized during secondary metabolic processes possessing immense potential as biological activity enhancers (Alves et al., 2024; Oszmiański et al., 2020).The toxicity of the plant extracts is assessed by this method.

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Received: March 27, 2022 – Accepted: November 9, 2022

Several advantages of this assay are rapidness, simplicity and low requirements. However, for standardized experimental conditions, several conditions need to be completed, especially (pH of the medium, temperature, aeration and light and salinity) (Hamidi et al., 2014). Certain anti-tumor and cytotoxic agents can be isolated from plants with the help of this bioassay and can be used against abnormal division of cells (Urmi et al., 2013). *Lemna minor* bioassay is helpful in investigating new plant growth stimulants (Hussain et al., 2010). With the increase in number of pests, management of pests becomes more complex and vice versa (Hyder et al., 2024). Weeds have adverse effects on the production of crops. For controlling these weeds different chemical herbicides are used. But these chemical herbicides cause environmental problems and are very expensive to use (Shahnoor et al., 2014). Therefore natural herbicides should be investigated which are safe and eco-friendly.

Interest in the use of therapeutic plants as insecticides has increased these days due to the environmental concerns and development of resistance to the synthetic insecticides in insects. These insects are one of the major causes of damage to fruits and vegetables throughout the world (Nazir et al., 2022). The search for attaining products from plants which may act as effective against certain plant diseases with less toxicity effect and less adverse effects on environment as well is being stressed upon by researchers (Dilkin et al., 2024). Plants contain the naturally occurring insecticides. Active constituents of plants are found to be effective against certain insects causing diseases in biological organisms (Santana et al., 2021). The mono-terpenoids are the bioactive agents present in medicinal plant extracts which cause mortality of insects. Due to their high volatile nature they have insecticidal activity which is useful for controlling stored-product insects (Ahmad et al., 2013).

Tribolium castaneum (red four beetle) is the main pest of flour and certain other products and has severely damaged stored grains like wheat (Suresh et al., 2001), affecting the quantity and quality of these food grains (Smith Junior et al., 1971). *Trogoderma granarium* (Khapra beetle) has caused major loss of stored grains in certain regions of Pakistan and is also one of the harmful insect pests (Bell and Wilson, 1995). Due to stored grains insect pests about 2- 6% food grains of Pakistan are lost each year during storage (Avesi, 1983). *Sphenoptera dadkhani* (Peach flat-headed borer) caused severe damage to the plum and peach orchards in Pakistan (Zahid, 2014). In Pakistan, yield of cotton crop decreases to about 28.13% due to pests (Javed et al., 2021). Potential negative effect of pesticides on pollinators has been a cause of concern too (El Helaly et al., 2021)

Achillea millefolium L. (Asteraceae) is known as Baranjasif, and its flowers and leaves are used medicinally (Ahmed, 2015). Plant has been used as antioxidant, antimicrobial, analgesic, anti-inflammatory, anti-hypersensitive, anti-diabetic, anticancer and anti-diarrheal (Presena, 2016). It is useful in hepatitis, jaundice and is a hepato-protective herb. It occurs in Azad Kashmir, Swat, Hazara and Kaghan (Fazal et al., 2013).

Chaerophyllum villosum L. (Apiaceae) is known as Jangali Gajar (Mehta and Bhatt, 2007). The leaves and seeds are used for the treatment of stomach pain, cough and cold (Aziz et al., 2015). It occurs at an altitude of (5000-6000 ft) (Khan et al., 2014). It grows in moist and cold environment on the road sides or open areas at height of (2100-3500 m) and is extensively distributed in East Asia Himalayas including India to Bhutan, Nepal and China (Joshi and Mathela, 2013). According to Flora of Pakistan it is found in the hills from (2500-4000m). The present study investigates the cytotoxic, phytotoxic and insecticidal activities of *Achellia millefolium* and *Chaerophyllum villosum*.

2. Materials and Methods

2.1. Plant materials

Both the plants *Achillea millefolium* and *Chaerophyllum villosum* were collected from Merajani top, Abbottabad District, Khyber Pakhtunkhwa, Pakistan at an altitude of (2,992 m) during August-September 2013.They were recognized by a taxonomist named Prof. Dr. Abdur Rashid at Botany Department, University of Peshawar, Pakistan. Voucher specimen numbers i.e. M. AdilBot.2244 (PUP) and M. Adil Bot. 2245 (PUP) were given and specimens were deposited in Herbarium, Botany Department, University of Peshawar. At room temperature plants were dried and ground with a mechanical grinder. The powdered plant materials (500g) were soaked in (1,000 ml), (97% methanol and chloroform) for two weeks. Both extracts were passed through (Whatman filter paper No.1823). The resulting methanolic and chloroformic extracts were subjected to rotary evaporator at 40ºC to get concentrated crude extracts. The aqueous extract was prepared by soaking 20 g powdered plant material in 80ml distilled water for 48 hrs. It was filtered to get the filtrate (Dastagir and Hussain, 2010).

2.2. Cytotoxic activity

The cytotoxic activity was done using the method of (Meyer et al., 1982). The stock solution was prepared when the methanolic, chloroformic and aqueous extracts of plant (10 mg) were dissolved in 1ml of dimethyl sulphoxide (DMSO)and then three concentrations i.e., 10µl, 100µl, 1,000µl were taken and were shifted to sterilized vials from this stock solution. At 85 ºC the vials were sterilized for 2 days. There were three replicates for each concentration. At low temperatures (4°C) the brine shrimp eggs were stored to maintain sustainability. The brine shrimp eggs were hatched in a tray (22x32 cm). It was half-filled with filtered brine solution (sea salt solution) and 50 mg eggs of brine shrimp was sprinkled and was subjected to incubation at 37°C.After 2 days the brine shrimp eggs hatched and 10 larvae/vial was placed. The volume was made to 10 ml with seawater and it was incubated at 25-27°C for 24 hours under illumination. In the other vials solvent was added, which served as negative controls. The (Etoposide) was used as standard drug and as a positive control.

2.3. Phytotoxic activity

Phytotoxic potential of the plant extracts was investigated against the *Lemna minor* by following McLaughlin (1991). In

(1,000 ml) distilled water several constituents were dissolved and the pH (5.5-6.0) was adjusted through addition of KOH pellets. The stock solution was prepared by dissolving extracts (30mg) in methanol and chloroform (1.0 ml). The three concentration 10 µg/ml, 100 µg/ml and 1,000 µg/ml, were taken from stock solution/standard solution and were shifted to Petri dishes (for each concentration 3 replicates were used). The petri dishes were left for sometime so that the solvents evaporate from Petri dishes. Then petri dishes were filled up with 20 ml E-medium and in each petri dish *lemna minor* plants with three fronds were added. The E-medium was added to other Petri dish for control. The initial reading of the *Lemna minor* bioassay was taken and then it was kept for a week. After a week the numbers of fronds in all petri dishes were counted and the data were arranged and were analyzed statistically (Steel et al.,1997). The following formula was used to calculate the percent inhibition of the *Lemna minor* (Equation 1).

$$\%\,inhibition = 100 - \frac{\text{Number of fronds in tests}}{\text{Number of fronds in negative control}} \times 100 \quad (1)$$

Insecticidal activity: The insecticidal activity was carried out as described by Bashir et al. (2010). The stock solution was prepared by mixing 20mg of the plant sample in 3ml of methanol and chloroform. In order to make the aqueous extract 20g of powdered plant was soaked in 80 ml of distilled water for 48 hrs. After 48 hrs it was filtered by means of standard filter paper. In controlled conditions of humidity and temperature (25-27ºC) the test insects such as *Tribolium castanium, Trochoderma granarium* and *Sphenoptera dadkhani* were raised in the plastic bottles. For insecticidal activity insects of same size and age were used. The insecticidal activity was done following (Bashir et al., 2010). On first day the petri dishes (90mm) were sterilized at 104 ºC for 4 hours and filter papers were shaped as per the dimensions of Petri plates. After that filter papers were placed in petri plates and then stock solutions of test samples were poured into it with micropipettes. The Petri dishes were left overnight for the evaporation of methanol and chloroform. On the second day ten small and equal sized healthy insects of each species were selected and were shifted to the labeled Petri dishes. In an incubator at 27°C in growth chamber the Petri dishes were placed for 24hrs with relative humidity of 50%. After 24 hrs incubation results were noted by calculating the number of survived insects in each Petri dish. Mortality percentage was calculated following the formula as follows (Equation 2).

$$\%\,mortality = 100 - \frac{\text{Number of insects in tests}}{\text{Number of insects in negative control}} \times 100 \quad (2)$$

2.4. Phytochemical screening

The phytochemical tests of methanolic, chloroformic and aqueous extracts of *Achillea millefolium* and *Chaerophyllum villosum* were done to find out tannins, glycosides, saponins, triterpenoids, phytosterols, phenols,, alkaloids, steroids, flavonoids and oils following the standard methods of Sofowora (1993), Trease and Evans (1989), Iyenger (1995), Kokate (2010), Edeoga et al. (2005).

3. Results and Discussion

3.1. Cytotoxic activity

The results revealed that different extracts from both plants showed dose dependent toxicity to brine shrimps. Methanolic extract of *Achellia millefolium* was most toxic to brine shrimps (73.4%) with LD_{50} value of 52.60 followed by chloroform extract (66.7%) at higher doses (Table 1). The result agrees with Naeem Qaisar et al. (2013) who

Table 1. Cytotoxic activity of *Achellia millefolium* and *Chaerophyllum villosum* Wall. ex DC.

Plant	Extracts	Dose (µg/ml)	Total no. of larvae	No. of survival larvae	No. of death of larvae	% mortality	LD_{50} (µg/ml)
Achillea millefolium	Control		30	30	0	0	-
	Methanol	10	30	19	11	36.7	52.60
		100	30	13	17	56.7	
		1,000	30	08	22	73.4	
	Chloroform	10	30	21	09	30.0	142.13
		100	30	16	14	46.7	
		1,000	30	10	20	66.7	
	Aqueous	10	30	25	05	16.7	385.39
		100	30	19	11	36.7	
		1,000	30	12	18	60.0	
Chaerophyllum villosum	Methanol	10	30	23	07	23.4	103.28
		100	30	15	15	50.0	
		1,000	30	07	23	76.7	
	Chloroform	10	30	16	14	46.7	14.81
		100	30	08	22	73.4	
		1,000	30	05	29	96.67	
	Aqueous	10	30	24	06	20.0	65.56
		100	30	11	19	63.33	
		1,000	30	01	29	83.4	

also reported lower LD_{50} value for methanolic extract of *Croton bonplandianum*. Similarly, chloroform extract of *Chaerophyllum villosum* measured maximum toxicity to brine shrimps (96.67%) with LD_{50} value of 14.81 followed by aqueous extract (83.4%) at higher doses (Table 1). These results are in accordance with Misonge et al. (2015) who also revealed highest toxicity of brine shrimps to the chloroformic extract of *Launaea cornuta.* This indicates that the toxicological activity shown by *A. millefolium* and *C. villosum* was due to the presence of cytotoxic agents. Earlier studies showed that saponins, alkaloids, tannins and flavonoids are some of the cytotoxic agents present in different plants (Huang et al., 2012; Mungenge et al., 2014).

3.2. Phytotoxic activity

The results revealed that methanolic extract of *Achellia millefolium* had showed profound growth inhibition (90.0%) of growth of *Lemna minor* with FI_{50} value of 6.60 µg/ml followed by chloroform (73.4%) and aqueous extract (63.4%) at higher doses (Table 2). The data also showed that methanolic extracts of *Chaerophyllum villosum* reduced the growth of *Lemna minor* by 76.7% with FI_{50} value of 0.67 µg/ml followed by chloroform (73.4%) and aqueous extract (70.0%).The methanolic extracts of *A. millefolium* and *C. villosum* caused greater growth inhibition of *Lemna minor* as compared to the chloroform and aqueous extract. The results are strengthened by the findings of Ghaffari et al. (2013) who also revealed maximum toxicity of *Lemna minor* plants due to methanolic extract of *Heliotropium dasycarpum*. Hameed et al. (2013) reported high toxicity of *Lemna minor* to methanolic extract of *Datura innoxia*. Different extracts of both the plants exhibited that % inhibition of the fronds of *Lemna minor* was dose dependent. Romero-Romero et al. (2002) reported that the phyto-toxins hamper the enzymatic activity, permeability of membrane, respiratory chains, division of cell and electron transport chain in photosynthesis. The herbicidal potential of both the plants might be due to the presence of phytotoxins.

3.3. Insecticidal activity

Results regarding insecticidal activity of different extracts of *Achellia millefolium* and *Chaerophyllum villosum* are shown in Table 3. The results indicated that chloroform extract of *Achellia millefolium* was most toxic (66.7%) to *Triboliumcastaneum* with LD_{50} value of 31.41 followed by methanolic extract (56.7%) at higher doses. Minimum toxicity (46.7%) was exhibited by aqueous extract at higher doses (Table 3). The methanolic extract of *Achellia millefolium* was most toxic (60.0%) to *Trochoderma granarium* with LD_{50} value of 242.22 followed by chloroform extract (50.0%) at higher doses. The low (40.0%) toxicity was measured by aqueous extract at higher doses. The study further revealed that methanolic extract of *Achillea millefolium* showed greater toxicity (90.0%) to *Sphenoptera dadkhani* (LD_{50} value of 4.17) followed by chloroform extract (80.0%) at high doses. Aqueous extracts of the same plant noted low (66.7%) toxicity to *Sphenoptera dadkhani* at high doses (Table 3). The methanolic extract

Table 2. Phytotoxic activity of *Achellia millefolium* and *Chaerophyllum villosum* Wall. ex DC.

Plants	Extracts	Dose (µg/ml)	No. of fronds in test	No. of fronds in control	% inhibition	FI_{50} (µg/ml)
Achellia millefolium	Methanol	10	13	30.00	56.7	6.60
		100	09		70.0	
		1,000	03		90.0	
	Chloroform	10	18		40.0	33.69
		100	12		60.0	
		1,000	08		73.4	
	Aqueous	10	20		33.4	105.75
		100	14		53.4	
		1,000	11		63.4	
Chaerophyllum villosum	Methanol	10	12	30.00	60.0	0.67
		100	09		70.0	
		1,000	07		76.7	
	Chloroform	10	15		50.0	7.80
		100	10		66.7	
		1,000	08		73.4	
	Aqueous	10	18		40.0	59.08
		100	15		50.0	
		1,000	09		70.0	

Table 3. Insecticidal activity of *Achillea millefolium* L.

Test Insects	Extracts	Dose (µg/ml)	Total No. of insects	No. of insects survival	No. of dead insects	Percent mortality	LD_{50} (µg/ml)
Triboliumcastaneum	Control		30	0	0	0	-
	Methanol	10	30	19	11	36.7	252.88
		100	30	16	14	46.7	
		1,000	30	13	17	56.7	
	Chloroform	10	30	16	14	46.7	31.41
		100	30	14	16	53.4	
		1,000	30	10	20	66.7	
	Aqueous	10	30	24	06	20.0	1228.97
		100	30	18	12	40.0	
		1,000	30	16	14	46.7	
Trochodermagranarium	Methanol	10	30	21	09	30.0	242.22
		100	30	17	13	43.4	
		1,000	30	12	18	60.0	
	Chloroform	10	30	25	05	17.7	838.68
		100	30	19	11	36.7	
		1,000	30	15	15	50.0	
	Aqueous	10	30	27	03	10.0	2397.07
		100	30	21	09	30.0	
		1,000	30	18	12	40.0	
Sphenopteradadkhani	Methanol	10	30	12	18	60.0	4.17
		100	30	09	21	70.0	
		1,000	30	03	27	90.0	
	Chloroform	10	30	15	15	50.0	10.14
		100	30	10	20	66.7	
		1,000	30	06	24	80.0	
	Aqueous	10	30	19	11	36.7	65.98
		100	30	13	17	56.7	
		1,000	30	10	20	66.7	

of *Chaerophyllum villosum* was most active (93.4%) against *Tribolium castaneum* with LD_{50} value of 8.90 followed by chloroform extract (90.0%) at high doses. The low (46.7%) mortality was exhibited by aqueous extract at high doses (Table 4). Methanolic extract of *Chaerophyllum villosum* was most toxic (83.4%) to *Trochoderma granarium* (LD_{50} value of 0.76) followed by chloroform extract (60.0%). The low (53.4%) toxicity was shown by aqueous extract at high doses (Table 4). Methanolic extract of *Chaerophyllum villosum* showed greater toxicity (83.4%) to *Sphenoptera dadkhani* with LD_{50} value of 0.34 followed by chloroform extract (80.0%) at high doses (Table 4).The methanolic extracts of *A. millefolium* and *C. villosum* showed maximum toxicity to *Sphenopteradadkhani*. In the present study the LD_{50} values for *Tribolium castaneum* and *Trochoderma granarium* were high and hence these insects showed resistance to the *A. millefolium* extracts. These results are supported by Hussain et al. (2010) who reported that *T. castaneum*and *T. granarium* were resistant to methanolic extract of *Rumex hastatus*. The growth inhibition of *Lemna minor* might be due to the occurrence of triterpenoids, glycosides, amino acids and saponins in these plants (Table 5). Similar results are also reported by Fazal et al. (2013) and Saleem et al. (2014). Mostafa et al. (2012), Gonzalez et al. (2013) and Jide-Ojo et al. (2013) reported that steroids, phenolics and flavonoids have shown toxicity against pathogens, pests and tested insects. Similar compounds were also present in the tested plants (Table 5). These results showed that different extracts from *A. millefolium* and *C. villosum* are potential sources of insecticides against the three tested insects. This also indicated that toxic phytochemicals are methanolic soluble that might be responsible for insecticidal activity.

Table 4. Insecticidal activity of *Chaerophyllum villosum* Wall. ex DC.

Test Insects	Extract	Dose (µg/ml)	Total No. of insects	No. of insects survival	No. of dead insects	Percent mortality	LD_{50} (µg/ml)
Triboliumcastaneum	Control		30	0	0	0	-
	Methanol	10	30	14	16	53.4	8.90
		100	30	08	22	73.4	
		1,000	30	02	28	93.4	
	Chloroform	10	30	20	10	33.3	86.61
		100	30	14	16	53.3	
		1,000	30	07	27	90.0	
	Aqueous	10	30	26	04	13.4	1407.7758
		100	30	21	09	30.0	
		1,000	30	16	14	46.7	
Trochodermagranarium	Methanol	10	30	11	19	63.4	0.76
		100	30	07	23	76.7	
		1,000	30	05	25	83.4	
	Chloroform	10	30	22	08	26.7	263.47
		100	30	17	13	43.4	
		1,000	30	12	18	60.0	
	Aqueous	10	30	23	07	23.4	685.38
		100	30	19	11	36.7	
		1,000	30	14	16	53.4	
Sphenopteradadkhani	Methanol	10	30	10	20	66.7	0.34
		100	30	08	22	73.4	
		1,000	30	05	25	83.4	
	Chloroform	10	30	17	13	43.4	25.02
		100	30	12	18	60.0	
		1,000	30	06	24	80.0	
	Aqueous	10	30	19	11	36.7	59.64
		100	30	14	16	53.4	
		1,000	30	08	22	73.4	

Table 5. Qualitative phytochemical screening of *Achillea millefolium* L. and *Chaerophyllum villosum*Wall. ex DC.

Chemical constituents	***Achillea millefolium* L.**			***Chaerophyllum villosum*Wall. ex DC.**		
	Methanolic extract	Chloroform extract	Aqueous extract	Methanolic extract	Chloroform extract	Aqueous extract
Alkaloids	-	+	+	+	+	+
Flavonoids	+	+	+	+	+	-
Phenols	+	+	-	+	+	+
Saponins	+	-	+	-	+	-
Glycosides	-	+	+	-	-	+
Tannin	+	+	-	+	-	+
Triterpenoids	-	+	+	-	-	-
Steroids	-	-	+	+	+	+

Note: (+) indicates the presence of phytochemical while (-) indicates absence of phytochemical.

4. Conclusion

The results concluded that different extracts from *A. millefolium* and *C. villosum* showed good cytotoxic, phytotoxic and insecticidal activity in a dose dependent manner. The cytotoxicity exhibited by the present plants clearly indicates the presence of potent bioactive compounds and they might be helpful in future for the treatment of cancer. The phytotoxic effect as shown by both the plants revealed that these plants are rich sources of phytotoxic compounds and might serve as a good source of natural herbicide for the control and management of weeds in agriculture in order to improve crops yield. The insecticidal activity of both plants indicated that plant extracts which are eco and user friendly, play vital role in protection of storage commodities. Therefore, these extracts may be potential candidates for their use in the

formulation of commercial repellents and insecticides that serve as effective control option, in the management of stored product insects responsible for huge loss of food commodities during storage.

Acknowledgements

The research was supported by The Deanship of Scientific Research (DSR) at King Abdul Aziz University, Jeddah, Grant No. [IFPRC-187-130-2020]. The authors are highly grateful for this support.

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