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Management of Fruit Rot of "*Bhut Jolokia*" (*Capsicum chinense*Jacq.) Caused by *Colletotrichum gloeosporioides* with Biological Approaches

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ABSTRACT
Fruit rot of 'bhut jolokia' caused by Colletotrichum gloeosporioides is a very serious disease prevalent in North East India predominantly in the states of Assam, Nagaland and Manipur. The present investigation is aimed at management of the disease by few bio-agents and botanicals in-vitro as well as in-vivo. Six bio-agents viz. Trichoderma viride, T.harzianum, T. pseudokoningii, T. asperellum, Paecilomyces lilacinus and Pseudomonas fluorescens were tested against C. gloeosporioides. Out of these, T. viride was found to be most effective followed by T. harzianum and T. pseudokoningii respectively. Twelve botanicals were evaluated at 50 percent concentration in-vitro for their efficacy against C. gloeosporioides by 'poisoned food technique'. Amongst the botanicals, Allium sativum showed highest inhibition of mycelial growth followed by Acorus calamus and Azadirachta indica. These botanicals were further tested against C. gloeosporioides at three different concentrations viz., 5 %, 10% and 15% respectively. Amongst these 15 % concentration was found superior to others. Based on in-vitro tests, three effective bio-agents and botanicals were tested against the disease in pot condition. Results showed that seed treatment and foliar spray of A. sativum was found to be most effective, showing lowest disease incidence (7.35%) and percent disease index (7.49). This was followed by seed treatment and foliar spray of A.calamus. Among the bio-agents, seed treatment and foliar spray with T.viride showed lowest disease incidence (17.24%) and percent disease index (17.56) compared to other bio-agents. Highest yield (113g/plant) was recorded in seed treatment and foliar spray with A. sativum followed by seed treatment and foliar spray with A. calamus (95g/plant). On the other hand, lowest yield was recorded in control (30g/plant). Spraying with plant extracts and antagonistic micro organisms were effective against C. gloeosporioides, but all these ranked behind the chemical fungicide Captan (0.2%).
Keywords: Biological management, bhut jolokia, biocontrol agents, botanicals, Colletotrichum gloeosporioides.

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INTRODUCTION

'Bhut jolokia', (*Capsicum chinense* Jacq.) is extensively grown in the North Eastern region of India predominantly in the States of Assam, Nagaland and Manipur. It has been acknowledged as the hottest chilli in the Guinness Book of World Records having a rating of 1,001,304 Scoville Heat units (SHU's). [1]. '*Bhut jolokia'* is consumed as green and fully ripe fruits, either raw or cooked with vegetables and also as spice. Even though, '*bhut jolokia'* is a very important spice in North Eastern India, the productivity is lower due to various diseases and pests. Among the diseases, fruit rot caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., is one of the most important disease causing severe damage to fruit in the field as well as in storage. Management of the disease with fungicide is not preferred due to bio-safety consideration. Indiscriminate use of fungicides for the management of the disease may lead to development of resistance to fungicides, residual toxicity, atmospheric pollution etc. Therefore, there is an urgent need of alternative control components which will be effective at farmers level. Biological management of the disease with bio-agents and botanicals is a distinct alternative possibility and eco-friendly approach for its management. With the above view points, the present research work was done to find out an approach using antagonistic microorganism and botanicals for effective management of fruit rot of '*bhut jolokia'* .

MATERIAL AND METHODS

Plant materials, pathogens and bio-agents

Diseased fruits of '*bhut jolokia'* (*Capsicum chinense* Jaqc.) showing typical symptoms of fruit rot caused by *Colletotrichum gloeosporioides* were collected from Jorhat. The pathogen was isolated on sterilized potato dextrose agar (PDA) medium. The pure cultures of the bioagents used for the present study were collected from the culture collections of the Department of Plant Pathology, AAU, Jorhat.

Efficacy of bioagents against *Colletotrichum gloeosporioides in-vitro*

Antagonistic effect of *Trichoderma harzianum*, *T. viride, T. pseudokoningii, T. asperellum, Pseudomonas fluorescens, Paecilomyces lilacinus* were tested against *Colletotrichum gloeosporioides* by dual culture method (Dennis and Webster, 1971). Mycelial discs (5mm diameter) of each bioagents were placed on one side of the Petri plates (9 cm diameter) near the periphery. In the opposite side of the Petri plates, a disc of *Colletotrichum gloeosporioides* was placed. The bacterial bioagents were placed on the periphery by streaking. The dual cultures assays were replicated five times and incubated at 28±1°C for 7 days. Plates without any test pathogen served as control. Based on the *in vitro* performance three effective bioagents were selected for further studies.

Preparation of plant extracts:

Different plant parts (leaves, shoots, bulbs and rhizomes) of twelve selected botanicals were collected and the aqueous extracts were prepared following the procedure of Shekhawat and Prasad [17] with certain modification. Fresh tissues of the plant species were ground along with sterile distilled water (@50g/50ml *i.e.* 1:1 w/v) in a pestle and mortar. The extracts were filtered through muslin cloth and finally through Whatman No 1. filter paper under aseptic condition. Then it was centrifuged at 10,000 rpm for 15 minutes and supernatants were used for *in vitro* studies. This formed the standard aqueous plant extract solution (100%).

Efficacy of botanicals against *Colletotrichum gloeosporioides in vitro*

The selected botanicals were first screened for their efficacy against the pathogen at 50% concentration with 5 replications by 'poisoned food technique' [12]. For this, 50ml of 100% aqueous extract of each botanical was mixed aseptically with 50ml molten PDA double strength medium and about 20ml of this medium was poured in each Petri dishes. One mycelial disc(5mm diameter) of the test pathogen was placed at the center of the Petri dish after solidification of the medium. The medium without plant extracts but with 50 ml sterilized distilled water served as control. The plates were incubated at 28±1ºC for 7 days and the percent inhibition of the mycelial growth was calculated by the formula of Vincent [19]. Based on the performance of preliminary screening, three most promising botanicals were selected and further tested against the pathogenat5, 10 and 15 % concentration.

Evaluation of plant extracts, antagonists and *fungicide for management of fruit rot disease under pot conditions*

A pot experiment was carried out at the Department of Plant Pathology, Assam Agricultural University, Jorhat, in a Completely Randomized Design with 5 replications for 22 treatments. The site of the experiment is situated at 26 °47′ N latitude, 94°12′ E longitude and at an elevation of 86.56 m from the mean sea level in the Upper Brahmaputra Valley Zone of Assam. Based on preliminary screenings, three promising bioagents and three promising botanicals at 15 per cent concentration were selected to carry out the pot experiment. The treatment combinations comprised of seed treatment (ST) or foliar spray (FS) or their combinations for both bioagents and botanicals which were compared with fungicidal treatments (Captan @ 0.2%) and untreated control.

For seed treatment with bioagents and botanicals 3ml of bioagents solution/extracts were used for 10g of seed. Seeds were soaked for 6 hrs and then shade dried for 2 hrs before sowing. Seeds were treated with 0.2% Captan (2g/litre) solution for 24 hours before sowing. The treated seeds (with bio-agents, botanicals and fungicide) and untreated seeds were sown in the nursery and 45 days old seedlings were transplanted in pots. Foliar spray with bioagents, botanicals and fungicide Captan were done at 120 days after transplanting of seedlings *i.e*. when the fruits were at the initiation of ripening stage. The second spray was done 20 days later.

Incidence of fruit rot of '*bhut jolokia'* was recorded 10 days after last spray and disease incidence (DI) was expressed in terms of percentage was calculated as follows

$$\text{DI (\%)} = \frac{\text{No diseased fruits/plant}}{\text{Total no of healthy and diseased fruits}} \times 100$$

The intensity of fruit rot disease was recorded by adopting the following rating [15].

The percent disease index was calculated using the McKinney's [9] formula

$$\text{PDI} = \frac{\text{Total number of numerical rating}}{\text{Total number of fruits observed}} \text{x} \frac{100}{\text{Maximum category value}}$$

Record on plant height, yield attributes and yield

The plant height (cm) was recorded at the time of fruit ripening stage. The average number of fruits harvested per plant was recorded. Five randomly selected fruit of a single plant was measured from the base of the pedicel to the tip of the fruit and the average was worked out and expressed in cm. Fruit girth was measured at the shoulder portion of the fruit and expressed in cm. Weight of individual fruit from plant was recorded by taking weight of 10 randomly selected fruit in each pot and average was calculated. Total weight of the fruits produced in each pot was recorded and average fruit yield per plant was calculated.

Statistical analysis

Fisher Method of analysis of variance was followed for statistical analysis of the experimental area. Significance of variance among the data were calculated out by calculating the 'F' value and comparing it with tabulated 'F' value at 5 per cent probability level. The percentage values were converted after Gomez and Gomez, 1984 and transformed by angular transformation. Different treatments were compared among themselves by calculating critical difference (CD) The significance and non-significance of the treatments at 5 per cent probability level were calculated out by multiplying the SEd with appropriate tabulated value for error degrees of freedom.

RESULTS AND DISCUSSION

In-vitro* assay of bio-agents against *Colletotrichum gloeosporioides

In vitro studies showed that *Trichoderma viride* recorded the highest inhibition of mycelial growth of the pathogen (70.22 per cent inhibition). It was followed by *T. harzianum*, *T. pseudokoninggi*, *T. asperellum* with 69.55, 59.11 and 58.66 per cent inhibition respectively over the untreated control. The antagonistic action of *Trichoderma* spp. against phytopathogenic fungi might be due to either by the secretion of extracellular hydrolytic enzymes. [2, 5, 16] or by the production of antibiotics [3, 4, 6]. Superiority of *T. viride* in inhibiting the mycelial growth of *Colletotrichum sp.* were reported by several workers. [8, 13].

Efficacy of different botanicals against *Colletotrichum gleoeosporioides*

The aqueous extracts of all the twelve botanicals at 50 per cent concentration were significantly effective in inhibiting the mycelial growth of *Colletotrichum gloeosporioides* in comparison to control. Among the botanicals bulb extract of *Allium sativum* was found to be the most effective in inhibiting the mycelial growth of the pathogen (91.11%), which was followed by *Acorus calamus* (88.55%) the effects of which were statistically *at par*. Next best was *Azadirachta indica* (84.88%) , showing statistically *at par* effect with that of *A. calamus*.

Based on results of this preliminary screening *Allium sativum, Acorus calamus, and Azadirachta indica* were selected for further studies. The effects of these three botanicals at 5, 10 and 15 % concentration on inhibition of mycelial growth of the pathogen resulted in decrease in mycelial growth with increase in concentration. However 15% concentration was found to be significantly superior on reducing the mycelial growth of the pathogen. The highest inhibitory effect of some of the tested extracts is correlated with the previous findings of Paramasivanm and Kalaimani [14] who tested 65 plant extracts (10%) for their efficacy against the mycelium growth, spore germination of *Colletotrichum capsici* causing fruit rot of chilli *in-vitro*. Several studies have reported the effectiveness of bulb extract of *A. sativum* in inhibiting mycelial growth of different fungi [13, 7]. The inhibitory action of *A. sativum* extracts may be due to the presence of volatile sulpher compounds garlicin, phytoncides-1and allicin which act as an inhibitor of respiratory –SH group enzymes [20].

Amongst the botanicals *A. sativum a*t 15 per cent concentration resulted in highest inhibitory effect (87.44%) on the mycelial growth (1.13cm) of the pathogen, over the control. This was followed by *A. calamus and A. indica* which resulted in 82.00 per cent and 73.77 per cent inhibition of mycelial growth of the pathogen, respectively over the control. The results of this investigation was in conformity with the findings of Shovan *et al* [18].

Table 1: Efficacy of different bio-agents on mycelial growth of *Colletotrichum gloeosporioides*

Treatments	Mycelial growth* (cm)	Per cent inhibition over control
T_1 : *Trichoderma pseudokoningii*	3.68	59.11
T_2 : *Trichoderma harzianum*	2.74	69.55
T_3: *Trichoderma asperellum*	3.72	58.66
T_4: *Trichoderma viride*	2.68	70.22
T_5: *Pseudomonas fluorescens*	3.88	56.88
T_6: *Paecilomyces lilacinus*	3.78	58.00
T_7 : Control	9.00	-----
SEd (±)	0.06	
CD(P=0.05)	0.13	

After 7days of incubation at 28±1°C
*Mean of five replications

Table 2: Efficacy of different botanicals (50%) on mycelial growth of *Colletotrichum gloeosporioides*

Treatments	Mycelial growth* (cm)	Per cent inhibiti over control
T_1: *Ocimum sanctum*	3.96	56.00
T_1: T_2: *Azadirachta ind*	1.36	84.88
T_3: *Aegle marmelos*	3.08	65.77
T_4: *Jatropha curcas*	3.16	64.88
T_5: *Cynadon dactylon*	3.32	63.11
T_6: *Cymbopogon nardus*	3.38	62.44
T_7: *Allium cepa*	2.92	67.55
T_8: *Allium sativum*	0.80	91.11
T_9: *Curcuma mangga*	2.00	77.77
T_{10}: *Curcuma amada*	2.04	77.33
T_{11}: *Acorus calamus*	1.03	88.55
T_{12}: *Tagetes erecta*	3.08	65.77
T_{13}: Control	9.00	------
SEd(±)	0.17	
CD(P=0.05)	0.35	

After 7 days of incubation at 28±1°C
* Mean of five replications

Table 3:Efficacy of effective botanicals on mycelial growth of *Colletotrichum gloeosporioides*

Treatments (Botanicals and concentrations)	Mycelial growth* (cm)	Per cent inhibition over control
B_1 : *Allium sativum*		
C_1= 5%	3.35	62.77
C_2=10%	3.14	65.11
C_3=15%	1.13	87.44
B_2: *Acorus calamus*		
C_1= 5%	4.65	48.33
C_2=10%	3.36	62.66
C_3=15%	1.62	82.00
B_3: *Azadirachta indica*		
C_1= 5%	5.20	42.22
C_2=10%	4.65	48.33
C_3=15%	2.36	73.77
Treatment Mean	3.29	----
Control	9.00	----
Botanicals (B)	SEd± CD (P=0.05)	----
Concentrations (C)	0.03 0.06	----
Control Vs Treatments	0.03 0.06	----
	0.04 0.07	----

* Mean of five replications

Table 4: Efficacy of bioagents and botanicals on yield attributes and yield of '*bhut jolokia*' in pot culture

Treatments	No. of healthy fruits/ plant	Weight of single fruit (g)	Fruit yield (g/plant)	Percent increase over control
T_1: ST with *T. viride*	12.40	5.24	65.08	45.91
T_2: ST with *T. harzianum*	11.00	5.14	56.40	26.45
T_3: ST with *T. pseudokoningii*	10.60	5.12	48.80	9.41
T_4: FS with *T. viride*	10.60	5.10	54.34	21.83
T_5: FS with *T .harzianum*	9.80	5.10	49.94	11.97
T_6: FS with *T. pseudokoningii*	9.40	5.10	50.82	13.94
T_7: ST+ FS with *T. viride*	13.20	5.02	66.30	48.65
T_8: ST + FS with *T. harzianum*	12.40	5.12	63.44	42.24
T_9: ST + FS *T. pseudokononggii*	9.60	5.00	48.00	7.62
T_{10}: ST with *A. sativum*	12.40	5.28	65.28	46.36
T_{11}: ST with *A.calamus*	11.60	5.16	59.76	33.99
T_{12}: ST with *A. indica*	10.00	5.04	50.46	13.13
T_{13}: FS with *A. sativum*	11.00	5.22	57.38	28.65
T_{14}: FS with *A. calamus*	13.00	5.06	65.66	47.21
T_{15}: FS with *A. indica*	13.00	5.02	65.26	46.32
T_{16}: ST+FS with *A. sativum*	22.60	5.68	127.60	184.75
T_{17}: ST+FS with *A.calam*us	19.00	5.10	96.90	117.26
T_{18}: ST+FS with *A. indica*	18.20	5.12	95.24	113.54
T_{19}: ST with Captan (0.2 %)	24.00	5.32	127.68	186.27
T_{20}: FS with Captan (0.2 %)	15.00	5.48	82.22	84.34
T_{21}: ST+ FS with Captan (0.2 %)	24.60	5.76	140.56	215.15
T_{22}: Control	8.80	5.04	44.60	-----
SEd±	0.94	0.13	4.90	-----
CD (P = 0.05)	1.56	0.21	8.13	-----

ST = Seed treatment FS = Foliar spray
All observations are record of first picking only

Table 5 : Efficacy of bio-agents and botanicals on growth and yield attributes of '*bhut jolokia*' in pot culture

Treatments	Plant height (cm)	Length of fruit (cm)	Girth of fruit (cm)	No. of total fruits/ plant
T_1: ST with *T. viride*	72.00	5.10	3.30	13.80
T_2: ST with *T. harzianum*	70.00	5.50	3.32	12.80
T_3: ST with *T. pseudokoningii*	72.00	5.10	3.22	10.00
T_4: FS with *T. viride*	74.00	5.08	3.04	11.80
T_5: FS with *T. harzianum*	73.00	4.50	3.08	11.40
T_6: FS with *T. pseudokoningii*	87.00	4.30	3.18	10.60
T_7: ST+ FS with *T. viride*	79.80	5.70	3.16	14.60
T_8: ST + FS with *T. harzianum*	73.80	4.36	3.10	13.80
T_9: ST + FS *T. pseudokononggii*	82.00	4.50	3.20	11.40
T_{10}: ST with *A. sativum*	80.20	5.14	3.12	13.60
T_{11}: ST with *A.calamus*	78.40	5.04	2.92	12.80
T_{12}: ST with *A. indica*	73.40	5.48	3.14	13.20
T_{13}: FS with *A. sativum*	86.00	4.46	3.16	12.20
T_{14}: FS with *A. calamus*	83.00	4.36	3.20	14.60
T_{15}: FS with *A. indica*	84.00	4.42	3.42	14.20
T_{16}: ST+FS with *A. sativum*	83.00	5.40	3.50	23.00
T_{17}: ST+FS with *A.calam*us	86.00	4.82	3.36	20.20
T_{18}: ST+FS with *A. indica*	84.00	5.62	3.20	19.20
T_{19}: ST with Captan (0.2 %)	70.00	5.38	3.30	25.00
T_{20}: FS with Captan (0.2 %)	82.00	5.12	3.14	16.00
T_{21}: ST+ FS with Captan (0.2 %)	74.00	5.50	3.34	25.00
T_{22}: Control	70.00	4.06	3.12	15.60
SEd±	2.62	0.20	0.10	0.95
CD (P = 0.05)	4.34	0.33	0.17	1.57

ST = Seed treatment FS = Foliar spray
All observations are record of first picking only

Table 6: Efficacy of bio-agents and botanicals on per cent disease incidence and disease intensity of fruit rot of '*bhut jolokia'* in pot culture.

Treatments	Disease incidence (%)	PDR over control	Per cent disease index	PDIR over control
T_1: ST with *T. viride*	9.60 (18.24)*	76.79	12.01 (20.46)*	72.64
T_2: ST with *T. harzianum*	14.30 (21.80)	65.57	14.10 (21.58)	67.88
T_3: ST with *T. pseudokoningii*	20.70 (26.80)	50.17	15.32 (22.81)	65.10
T_4: FS with *T. viride*	10.60 (20.86)	74.48	15.06 (22.81)	65.69
T_5: FS with *T .harzianum*	17.20 (24.66)	58.59	24.25 (29.78)	44.76
T_6: FS with *T. pseudokoningii*	20.60 (28.28)	50.41	13.30 (20.74)	69.70
T_7: ST+ FS with *T. viride*	9.06 (17.37)	78.36	14.76 (22.54)	66.38
T_8: ST + FS with *T. harzianum*	10.12 (18.73)	75.64	8.50(17.21)	80.64
T_9: ST + FS *T. pseudokonongii*	16.13 (23.83)	61.17	16.88 (24.23)	61.55
T_{10}: ST with *A. sativum*	9.07 (17.36)	78.16	24.58(29.72)	44.01
T_{11}: ST with *A.calamus*	9.39 (18.04)	77.39	26.34 (29.82)	40.00
T_{12}: ST with *A. indica*	16.29 (24.01)	60.78	18.23 (25.22)	58.47
T_{13}: FS with *A. sativum*	9.87 (17.83)	76.24	10.10 (18.56)	76.59
T_{14}: FS with *A. calamus*	15.20 (22.61)	63.41	10.60 (18.35)	75.85
T_{15}: FS with *A. indica*	16.02 (23.33)	61.43	10.62 (18.62)	75.81
T_{16}: ST+FS with *A. sativum*	2.50 (7.85)	94.07	2.30 (7.60)	94.76
T_{17}: ST+FS with *A.calam*us	5.80 (14.34)	86.04	5.90 (13.92)	86.56
T_{18}: ST+FS with *A. indica*	5.20 (13.81)	87.48	5.58 (13.61)	87.29
T_{19}: ST with Captan (0.2 %)	4.00 (11.54)	90.37	8.00 (16.43)	81.77
T_{20}: FS with Captan (0.2 %)	7.44 (16.36)	82.09	6.28 (14.09)	85.69
T_{21}: ST+ FS with Captan (0.2%)	1.52 (6.80)	96.34	1.90 (7.04)	95.67
T_{22}: Control	41.54 (44.03)	------	43.90 (41.65)	-----
SEd±	2.30	------	2.00	------
CD (P = 0.05)	3.82	------	3.33	------

ST = Seed treatment FS = Foliar spray

* Figures in parentheses are angular transformed values.

PDR= Per cent disease reduction PDIR= Per cent disease index reduction

Efficacy of bioagents and botanicals on growth, yield attributes and yield of '*bhut jolokia'* in pot culture

Application of foliar spray (FS) of *Trichoderma pseudokoningii* resulted in significantly higher plant height of (87.00 cm) which was followed by foliar spray with *Allium sativum* (86.00 cm) and ST+FS with *Acorus calamus* (86.00 cm). The lowest plant height was recorded under untreated control (70.00 cm).

Amongst the bioagents ST+FS with *T. viride* (5.70cm) resulted in highest fruit lengths. Similarly, amongst botanicals the effect of ST+FS with *A. indica* (5.62cm) was highest. Girth of fruits was highest in ST+FS with *A. sativum* (3.50cm), the effect of which was *at par* with the treatments ST+FS with *A. calamus* (3.36cm) and ST+FS with Captan (3.34cm). In case of number of fruits per plant, results showed that combined application of botanicals as seed treatment and foliar spray resulted in similar effects as that of ST+FS with fungicide(25). The number of healthy fruits per plant was significantly higher under the treatment ST+FS with Captan (24.60), the effect of which was statistically *at par* with the values under the treatment ST with Captan (24.00). Amongst the bioagents, ST+FS with *T. viride* resulted in higher number of healthy fruits (13.20). Combined methods of application of ST+FS with Captan produced significantly the highest values of fruit weight (5.76g/fruit), which was statistically at par that under values of ST+FS with *A. sativum* (5.68g/fruit). ST+FS with Captan resulted in highest fruit yield of 140.56 g/plant over rest of the treatments resulting 215.15% increase in yield over the control. This was followed by the treatments ST+FS with *A. sativum* (127.60 g/plant) and ST with Captan (127.68 g/plant), the effects of which were statistically at par, producing 184.75 and 186.27 percent increase in yield over the control, respectively. Amongst the bioagents, ST+FS with *T. viride* (66.30g/plant) and ST with *T. viride* (65.08g/plant) resulting in higher fruit yield with 48.65 and 45.91 per cent increased yields, respectively over the control. Similar results were achieved by Muthukumar [10] where seed treatment and foliar spary with *T. viride* was superior in reducing the pre and post-emergence damping-off incidence and increased the plant growth and yield of chilli when compared to control in pot culture.

Efficacy of bioagents and botanicals on per cent disease incidence and disease intensity of fruit rot of '*bhut jolokia'* in pot culture

Highest disease reduction (96.34%) was recorded with Captan (T_{21}) when applied in combination of seed treatment (ST) and foliar spray (FS), which was followed by ST+FS with *A. sativum* (T_{16}= 94.67 %) the

effects of which were statistically at par. Highest reduction in PDI was recorded through ST+FS with Captan (T_{21}= 95.67%, which was followed by ST+FS with *A. sativum* (T_{16} = 94.76%), the effects of which were significantly *at par* to each other. Maximum disease reduction was obtained when the treatments were given as combination of ST and FS. *In vitro* and *in vivo* evaluation showed greatest reduction of disease severity by *A. sativum* at 5% concentration [11].

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