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Short Research Article

Salivia moorcroftiana a New Host for *Sclerotium rolfsii* Sacc. in India–its Management with Botanicals and Fungicides

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Abstract

Salvia is the largest genus of plants in the mint family and is one of the important medicinal plants. *Salvia* spp. are valued for essential oil, flavor compounds and their possible uses have been registered as an antisweating agent, antibiotic, antifungal, astringent, antispasmodic, estrogenic, hypoglycemic and tonic agents. In the present investigation the specie, *Salvia moorcroftiana* was for the first time found infected with *Sclerotium rolfsii* Sacc. in Himachal Pradesh (India) causing typical crown rot symptoms, the losses of which amounted upto 25% in plantation areas surveyed during June-September 2011−12. Initially the symptoms appeared as yellowing and drooping of leaves proceeded by browning and later the leaves get distorted with wilting of plants and white cottony mycelial growth at the collar region. The mycelial growth spread to the stem and roots, with associated tissue rotting. The morphological characters of the crown rot causing fungus resembles to *Sclerotium rolfsii.* The botanicals and fungicides were considered for possible management of the disease. Out of eight plant leaf extracts, only three *Melia azedarach, Bougainvellia* and *Aloe barbidensis* showed high efficacy and recorded maximum inhibition of mycelial growth of the test pathogen (*S. rolfsii*) by 62.80, 58.61 and 55.34% over control under *in vitro*. Similarly fungicides, chlorothalonil, propineb, carbendazim and benlate not only reduced the disease incidence but improved the seed germination of the infected seeds and final plant stand with minimum pre- and post-emergence mortality rate hence can be recommended in managing the crown rot disease in *Salvia moorcroftiana*.

1. Introduction

Salvia referred to "sage" is derived from the Latin *salvere* (to save), referring to the healing properties long attributed to the various *Salvia* species. *Salvia moorcroftiana* Wall, belonging to the family Lamiaceaea is commonly called as 'kallijari' is a native to the Himalayan mountains stretching from Pakistan to western Nepal where it grows between 5,000−9,000 ft elevation particularly on disturbed areas and open slopes (Li et al., 2013) . The plant is introduced to Himachal Pradesh due to multifarious medicinal properties showing antitumor activity, emetics, hemorrhoids which are present in leaves, seeds and roots (Singh and Thakur, 2014). The leaves serve as agents in controlling guinea worm and itching, and in the form of a poultice they are applied to wounds (Baser et al., 1998; Hayet et al., 2007). Some of their constituents revealed the presence of diterpenoids and flavonoids (Lu and Foo, 2002; Muhmmad Zahid, 2002). However, the *Salvia* species are susceptible to many kinds of diseases of which crown, collar or root rot are most serious. Diseases are major constraints to its production, the most serious and devastating is crown rot of *Salvia* species considered to be an economically important disease observed recently in plantations of the Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P). *Sclerotium rolfsii* Sacc. was found associated with crown rot in *Salvia moorcroftiana*. The fungus is also responsible for inducing various kinds of symptoms like damping-off, collar rot, root rot etc. in a wide range of crops including medicinal plants, thereby inflicting heavy losses under congenial environment conditions. The fungus is polyphagous and is reported to be soil borne in nature and is able to survive in soil years together forming dormant structures called sclerotia which are difficult to manage through cultural (Gilbert, 1991; Raynals et al., 1991) and chemical (Rohodes et al., 1992) means. Therefore the efficacy of various plant extracts and fungicides antifungal properties of plant extracts and fungicides efficacy was studied under *in vitro* and pot culture experiments to see their impact

in managing crown rot of *Salvia moorcroftiana,* effect against *Sclerotium rolfsii* progress.

2. Materials and Methods

Salvia (*Salvia moorcroftiana*) is an important medicinal crop grown in India. Between June and September, 2011 disease survey was conducted in different plantation areas in medicinal and aromatic research farm of the Dr. Y.S.Parmar University of Horticulture and Forestry, Nauni, Solan (H.P) State. A fungus was consistently isolated from diseased roots plated on potato dextrose agar medium and incubated at 25±2 °C. To confirm pathogenicity, 25−30 day old seedlings were planted in pots (7 cm dia.) containing 100 parts of sterilized soil and 1 part of mycelial inoculum, using an isolate of *S. rolfsii* from salvia multiplied in a sand:maize meal medium prepared in 1:3 ratio of their composition. The pots were kept at 25±2 °C with soil moisture content maintained at 70%. The pressure plate method (Mishra and Ahmed, 1987) was adopted to find the field capacity (FC) and permanent (FC) and permanent wilting point (PWP) of the soil, in order to calculate the available soil moisture (ASM), the formula ASM (%)=FC-PWP was used. The soil was sieved and oven dried for 24 h at 105 °C. The dried soil was mixed with the fungus as stated above on the sand/maize medium and transferred to the pots and sprinkled with water for the three days to get the fungus established in soil. Five seeds of *Salvia moorcroftiana* were sown in each pot having five replications. The weight of each pot was taken after adding the required water for maintaining soil moisture level at 70%, considered most appropriate for pathogen spread. The required soil moisture (%) of available soil moisture (%) was calculated as per the formula: Required soil moisture (%) of available soil moisture (%)=FC-PWP×Required soil moisture (%)+PWP. The quantity of water to be added in each pot was further calculated as follows: Water to be poured (ml)=Required soil moisture (%) of available soil moisture (%)×Weight of the pot (g). The plants were assessed 30 days after planting.

Inoculated plants developed typical symptoms on leaves, stem and roots, and *S. rolfsii* was consistently re-isolated. The infected plant parts stem and roots were washed under running water thoroughly, later soaked in the folds of blotting papers and cut into small bits (2 mm) in size from the junction of diseased and healthy portion of plant parts with the help of sterilized blade. The bits were surface sterilized by dipping in 0.1% mercuric chloride solution (Mohammed, 1962) for 10−15 seconds and then repeatedly washed thrice with sterilized distilled water to remove the traces of mercuric chloride and placed on sterilized filter paper to remove excess moisture. Later the bits were transferred to potato dextrose agar (PDA) slants under aseptic conditions in Laminar Air Flow chamber and the slants were incubated at 27 °C in B.O.D incubator for 72−96 hrs. The axenic culture of pathogen was further purified by hyphal tip method to confirm its identity. The cultural and morphological characteristics of the isolated fungus were examined critically under microscope. Based on the mycelium characters and sclerotia formation, this was identified by following the key as discussed by Barnett and Hunter (1972).

The pathogenicity was further confirmed to prove the Koch's postulates. The fungus was multiplied on the sand-maize medium by soaking the crushed maize seeds overnight in water. These were boiled just to get them soften. The excess of water was drained out and grains were air dried. The grains were then mixed with sand in 3:1 proportion along with 2% sucrose. The mixture (150 g) was filled in poly-propylene bags which were plugged with non-absorbent cotton and wrapped with paper. The bags containing mixture were sterilized in an autocalve at 15 lbs psi for 20 minutes. The sterilized medium was further inoculated with a week old culture of *Sclerotium rolfsii*. Three bits of 4 mm size of the test fungus were transferred into each poly-propylene bags and were incubated at 25 °C for 10−12 days for mass multiplication and use in pathogenicity test. The five sterilized plastic pots 6 inches in diameter were filled with formalin (5%) sterilized garden soil. Thereafter, 20 g of sand:maize meal medium containing fungus growth was placed in these pots about 8−10 cm deep three days before sowing the seeds of the *Salvia moorcroftiana*. In each pot 5 seeds were sown while the pots of control treatment were without the addition of the test fungus. The plants emerged from the seeds were examined regularly for the disease appearance. The symptoms were noticed on inoculated as well as uninoclated plants and it was observed that the symptoms become evident on inoculated plants while uninoculated plants did not show any symptoms. The fungus was reisolated and identified for confirming the pathogenicity.

2.1. In vitro evaluation of plant extracts

The test fungus, *Sclerotium rolfsii* was isolated from collar region of the infected Salvia plants during monsoon season when the infection was quite high due to prevailing optimum temperature and moisture levels. The pure culture of the pathogen was obtained by standard procedure, identified and maintained on sand: maize meal (20%) medium and used for soil inoculation. Cold water extracts of fresh leaves of eight plant species viz; Parthenium (*Parthenium hysterophorus*), Darek (*Melia azedarach*), Aloe (*Aloe barbadensis*), Anola (*Phyllanthus emblica*), Artemisia (*Artmissia annua*), Mentha *(Menthe piperata*), Bhang (*Cannabis sativa*), Bougainvella (*Bougainvellia glabra*) were prepared by homogenizing with sterile distilled water @ 1:1 (w/v) in a sterilized pestle and mortar and filtered through sterilzed muslin cloth , respectively

giving 100% plant extract solution which was heated at 40 °C for 10−15 min. in hot water bath to avoid contamination and were diluted to required concentrations with sterile distilled water for further studies. All the eight plant extracts (30%) of six plant species were evaluated *in vitro* against the test pathogen using poisoned food technique (Falck, 1907; Alice, 1984) applying completely randomized design along with three replications. Control constitutes test pathogen inoculation on PDA without plant extract addition. All the Petri plates were incubated at room temperature (26±2 °C). Observations on radial growth of the test fungus were recorded at 48 and 72 hrs after inoculation and per cent inhibition of test fungus was calculated using the formula: I=(C-T)/C×100 where, I=Per cent inhibition in growth of test pathogen, C=Radial growth of pathogen in control, T=Radial growth of pathogen in treatment (Nene and Thapliyal, 1993).

2.2. Pot culture assay of plant extracts

An experiment was set up in pot culture to test the antifungal effect of all the leaf extracts *in vitro* against the pathogen, *S.rolfsii*. The experiment was designed in completely randomized pattern and replicated thrice. Earthen pots were disinfected with 5% solution of $CuSO_4$ and filled with autoclaved potting mixture of soil: sand: FYM (2:1:1) and inoculated with mass culture of test pathogen @ 50 g kg^{-1} soil, watered lightly and allowed to multiply for a week. The various plant extracts selected @ 250 ml pot^{-1} were poured onto the sick soil and pot containing sick soil without drenching of any extract served as control. The surface sterilized seeds of *S. moorcroftiana* with sodium hypochloride (5%) were sown @ 10 seeds pot^{-1} watered lightly and kept in the polyhouses for further studies. Observations were recorded on seed germination, pre and post emergence mortality and final plant stand.

2.3. Screening of fungicides efficacy in vitro

Eight fungicides namely thiram (0.3%), copper oxychloride (0.3%), zineb (0.3%), chlorothalonil (0.2%), Propineb (0.2%), carbendazim (0.1%) and benlate (0.1%) thiaphenate methyl (0.1%) were evaluated against *S. rolfsii* under *in vitro* by poisoned food technique at recommended concentrations 1000 ppm. The radial growth of the pathogen was measured after every alternate days upto a week and per cent growth inhibition was calculated as per the Vincent (1947). These fungicides were tested for their potential as seed dresser in earthen pots (10 cm diam). The pots were filled with pre sterilized formalin (5%) soil mixture (soil: sand: FYM (farm yard manure) at ratio 2: 1:1), later infested with *S. rolfsii* @ 50 g kg^{-1} of soil and seeds treated with fungicides with their respective concentrations as mentioned in the Table 3 were sown @ 10 seeds pot^{-1} in three replications.The selected fungicides were dissolved separately in 100 ml of water in a beaker (200 ml) and seeds of *Slavia moorcroftiana* were treated thoroughly by dipping for two hours in the suspension of each fungicides. Later the suspension was drained off and the seeds were dried on blotting paper for 24 h and sown in the pots. Observations on germination, pre and post–emergence mortality and final plant stand were recorded.

3. Results and Discussion

3.1. Symptoms and morphology of pathogen

A typical crown rot symptoms were observed during July–Aug, 2011on *Salvia moorcroftiana*. A new soil borne disease showing crown rot was observed for the first time on 2-month old salvia plants in all areas surveyed resulting into 22–25% incidence. Symptoms first appeared as yellowing and drooping of leaves, browning of the infected plant leaves which later get distorted with wilting of plants and white cottony mycelial growth at the collar region. The mycelial growth spread to the stem and roots, with associated tissue rotting. On the diseased areas, brown sclerotia were observed. The mycelium of the fungus was hyaline, profusely branched showing clamp connections and septate. The abundant sclerotia were found associated with the crown and root region which were round to oblong, initially white and later brown, with an average diameter of 0.5–1.5 mm. The first signs of infection, though usually undetectable, are dark-brown lesions on the stem at or just beneath the soil level; the first visible symptoms are progressive yellowing and wilting of the leaves. Following this, the fungus produces abundant white, fluffy mycelium on infected tissues and the soil. *Sclerotia* of relative uniform size are produced on the mycelium: roundish and white when immature then becoming dark brown to black. Mature sclerotia resemble mustard seed. The fungus occasionally produces lesions under humid conditions. The symptoms and morphological characters of the crown rot causing fungus resembles to *Sclerotium rolfsii* which were similar to the published descriptions records of *Sclerotium rolfsii* given by Mordue (1974).The pathogen was identified as *Sclerotium rolfsii* based on its mycelial and sclerotial characters (Barnett and Hunter, 1972). Similar reports were given by Subramanayan (1964); Mohan et al. (2000). The prevalence of other soil borne diseases such as *Fusarium* spp or *Rhizoctonia solani* were also noticed on clary sage by Subbiah et al. (1996). Chang et al. (1997) observed a stem rot disease of stevia for the first time in India and identified the causal agent as *Sclerotinia sclerotiorum*. Megeji et al. (2005) recorded a stem rot disease on stevia at Palampur, Himachal Pradesh while Kamalakannan et al. (2006) reported root rot infection on stevia from Tamil Nadu. However, there was no report of crown rot (*Sclerotium rolfsii Sacc.*) infecting *Salvia moorcroftiana,* therefore the pathogen is reported to be new to this host responsible to induce crown rot in Himachal Pradesh, India.

3.2. Plant extracts and fungicides in managing crown rot

The results related to plant extracts revealed that all the plant extracts in comparison to control inhibit the mycelial growth of *Sclerotium rolfsii* (Table 1). Leaf extracts of *Melia azedarach, Bougainvellia* and *Aloe barbadensis* showed high efficacy and recorded maximum inhibition of mycelial growth of the test pathogen (*S. rolfsii*) by 62.80, 58.61 and 55.34% respectively over control, whereas minimum (30.72%) growth inhibition

Table 1: Impact of different plant extracts on the growth of *S. rolfsii* (*in vitro*)

Plant extracts	Germination (%)	Mortality(%)		Plant stand (%)
		Pre	Post	
Parthenium hysterophorus (Parthenium)	76.67 (61.20)	13.33 (21.10)	32.22 (33.88)	68.89 (56.11)
Aloe barbadensis (Aloe)	80.00 (63.91)	15.57 (22.64)	16.67 (23.46)	78.89 (62.69)
Melia azedarach (Darek)	86.67 (68.83)	10.00 (18.27)	8.89 (17.11)	81.11 (64.23)
Phyllanthus emblica (Anola)	63.67 (52.76)	22.22 (28.06)	26.67 (30.90)	60.00 (50.79)
Artemisia annua (Artimisia)	60.00 (50.76)	30.00 (33.18)	16.67 (23.19)	61.11 (51.41)
Mentha piperata (Mentha)	66.67 (54.76)	23.33 (28.06)	30.33 (33.18)	56.67 (44.98)
Cannabis sativa (Bhang)	70.00 (56.98)	35.55 (36.57)	36.67 (37.24)	50.00 (44.98)
Bougainvillea glabra (Bougainvillea)	83.33 (66.12)	21.11 (27.32)	21.11 (27.05)	80.00 (63.46)
Control (without plant extract)	56.67 (56.67)	55.50 (48.28)	56.00 (48.83)	35.67 (36.66)
SEm±	3.87	2.94	4.02	1.67
CD (p=0.01)	7.12	6.91	8.93	4.40

Mean of three replications; Figure in parenthesis are arc sine transformed values

was obtained in *Mentha piperata* followed by *Artemisia annua* (33.84%) and *Phyllanthus emblica* (33.54%). The leaf extracts of *Melia azedrachta, Aloe barbedensis* and *Bougainvellia glabra* under pot culture were found most efficacious in increasing seed germination, reducing pre as well as post emergence mortality and increasing percentage of plant stand (Table 2). However, maximum germination of 80.0–86.67% was recorded in *Melia azedarach, Aloe barbedensis* and *Bougainvellia glabra* as all theseplant extracts found statistically at par in their efficacy. *Parthenium hysterophorus* and *Cannabis sativa* were found next best treatments and registered germination within 70 to 76.67%. There is reduction in seedling emergence pre and post mortality percentage, minimum (10.0, 8.89%) being obtained in *Melia azedarach* followed by other three, *Parthenium hysterophorus*, *Aloe barbadensis*, *Artemisia annua* within the range of 13.33–16.67%, respectively. All the treatments were reported to improve the plant stand in comparison to untreated plants. Above Around 78.89 to 81.11% plant stand percentage was obtained in the three plant extracts to seed in comparison to 35.67% plant stand recorded in control. Three plant extracts, *Melia azedarach, Bougainvellia* and *Aloe barbadensis* were found superior due to the antifungal activity *in vitro* and under pot cultures by giving maximum inhibition of the pathogen mycelium growth as well as enhances the seedling emergence rate improves the mortality rate and the germination percentage. According to Jagtap and Kamble (2010) 10% alcoholic leaf extracts of *Melia azedarach*, *Clerodendruminerme* sp., *Hyptissuaveolens* sp. and *Swieteniam acrophylla* completely inhibited growth of both the sensitive and resistant isolates of *Sclerotium rolfsii*. Out of twenty different aqueous extracts of plant species, *Azadirachta indica* (73.8%) followed by *Cassia fistula* (73.5%) and *Cannabis sativa* (67.1%) gave maximum inhibitory effect on mycelial growth of *Sclerotium rolfsii* causing southern Sclerotium rot in sugar beet (Muhammad et al., 2010). Seeds treated with neem leaf, garlic clove and allamonda leaf extracts gave maximum suppression of damping-off pathogens of solanaceous crops and also resulted in improving of the seed germination and growth characters of tomato, eggplant and

Table 2: Effect of plant extracts on germination and mortality percentage caused by *S. rolfsii* in pot culture

Plant extracts	Colony diameter (mm)	Inhibition (%)
Parthenium hysterophorus (Parthenium)	52.75	41.38 (40.04)
Aloe barbadensis (Aloe)	40.15	55.34 (47.98)
Melia azedarach (Darek)	33.48	62.80 (52.65)
Phyllanthus emblica (Anola)	57.00	35.66 (36.62)
Artemisia annua (Artimisia)	60.40	33.84 (35.52)
Mentha piperata (Mentha)	62.35	30.72 (33.57)
Cannabis sativa (Bhang)	51.00	43.33 (41.16)
Bougainvillea glabra (Bougainvillea)	37.25	58.61 (49.82)
Control (without plant extract)	90.00	-
SEm±	0.35	0.78
CD (p=0.01)	1.27	1.52

chilli seedlings. The highest seed germination of tomato (86.67 %), eggplant (86.33%) and chilli (90.33%) was obtained in in seeds treated with neem leaf extract. Islam and Faruq (2012) also found neem leaf extract most effective against damping-off of tomato than other extracts. Amin et al. (2013) selected different plants viz., rhizome ginger, neem leaf, tobacco leaf, rhizome of turmeric, and cow's urine and found their effect against *Sclerotium rolfsii* at only higher concentration, while garlic clove, allamonda leaf, ginger rhizome, neem leaf, kalijira seed, turmeric rhizome, bel rhizome of turmeric inhibited the growth at a low level as reported by Sab et al. (2014). Among the botanical treatments, *Litsea citrate* seed extract was found to give effective control against the pests of peas. Crude stem extract of *Costus speciosus* and seed extract of *Chenopodium ambrosioides* were also found to be effective in reducing the pests of rice as investigated by Shitiri et al. (2014). Butt et al. (2016) reported similar inference with respect to plant extracts of indigenous plants like *Alstonia scholaris* and *Azadirachta indica* in managing *S. rolfsii* under *in vitro* condition at different concentrations (1%, 2%, 3%, 4% and 5%).

The results represented in Table 3 showed complete inhibition of the test fungus by chlorothalonil, propineb and carbendazim at recommended concentrations considered under study after 7 days of inoculation indicating cent per cent control. This was followed by thiophanate methyl and benlate which gave 6.33 and 10.50 mm colony diameter. The date further indicated that the germination was enhanced upto 82.22 and 80.0% in chlorothalonil, propineb and carbendazim treated seeds

Table 3: Effect of fungicides on radial growth of *S. rolfsii*, germination, mortality and final plant stand of *Salvia moorcroftiana* infected seeds

Fungicides	*In vitro* (Radial growth mm)	Inhibition (%)	Germination (%)	Mortality (%)		Total plant stand (%)
				Pre	Post	
Thiram (0.3%)	28.0	68.89	67.78 (55.40)	28.89 (32.44)	34.44 (35.89)	40.00 (38.84)
Copper oxychloride (0.3%)	37.0	53.89	45.55 (42.42)	66.66 (54.88)	22.22 (28.01)	33.33 (35.21)
Zineb (0.3%)	25.0	72.22	53.33 (46.90)	47.78 (43.78)	36.67 (37.17)	43.33 (40.76)
Chlorothalonil (0.2%)	0.0	100.0	82.22 (65.13)	17.78 (24.20)	22.22 (28.10)	76.66 (61.20)
Benlate (0.1%)	10.50	88.33	74.44 (59.69)	15.57 (22.69)	34.45 (35.86)	66.67 (54.76)
Propineb (0.2%)	0.0	100.0	80.00 (65.13)	17.78 (24.53)	14.45 (21.87)	80.00 (63.91)
Carbendazim (0.1%)	0.0	100.0	80.00 (63.46)	22.22 (27.35)	31.11 (33.84)	73.33 (58.98)
Thiophanate methyl (0.1%)	6.33	100.0	70.00 (56.79)	36.67 (37.24)	37.78 (37.88)	63.33 (52.76)
Control	90.0	0.0	40.00(39.00)	82.22 (65.06)	90.00 (71.70)	36.67 (37.21)
SEm±	--	--	2.03	5.42	3.97	5.03
CD (p=0.05)	--	--	5.89	11.36	6.70	11.60

followed by benlate where the germination % was recorded upto 74.44 %. The pre and post emergence mortality in different fungicides differed significantly but found statistically superior to untreated soil (inoculated with test fungus only). The per cent seedling emergence was significantly less (15.57–17.78%) and (14.45–22.22%) in chlorothalonil and propineb followed by carbendazim, benlate and copper oxychloride which directly helped in improving the plant stand. Higher plant stand of 80% was obtained in order of propineb, chlorothalonil (76.67%), carbendazim (73.33%) and benlate (66.67%).

The germination was enhanced upto 82.22 and 80.0% in fungicides chlorothalonil, propineb and carbendazim treated seeds followed by benlate besides restricting the pathogen growth under laboratory conditions. Similar results were obtained by Chauhan (1978) with regards to carbendazim which inhibited the growth of *S. rolfsii* maximum *in vitro*. There was improvement in the plant stand within the range of 66.67 to 80.0%. The present findings are in conformity with the results obtained by Randon et al. (1995); Tripathi and Khare (2006); Chakraborty and Bhowmik (1985). The fungicides chlorothalonil (0.2%), propineb (0.2%), benlate (0.1%) in addition to carbendazim (0.1%) and thiram (0.1%) possessed high fungitoxicant effect in restricting the growth of the fungus. Wang et al. (2015) found that fluazinam has potent antifungal activity against *Sclerotium rolfsii* which causes stem rot. Soil fumigant such as methyl bromide, methane sodium and chloropicrin inhibits the growth of mycelium of *S. rolfsii*. The radial growth of *S. rolfsii* under *in vitro* conditions was also inhibited maximum with four fungicides tegula (tebuconazole), thiophanate methyl, ridomil gold (metalaxyl+mancozeb) and mancozeb significantly (Khan and Javaid, 2015).

4. Conclusion

Plant extracts of different species particularly *Melia azedarach, Bougainvellia* and *Aloe barbadensis* if applied @ 30% and fungicides chlorothalonil, propineb and carbendazim at

their recommended concentrations of 0.2% can successfully managed the crown rot disease of *Salvia moorcroftiana.* Both the seed as well soil drench treatment of fungicides can be used alone or along with application of plant extracts which probably will reduce the cost on fungicide application in the integrated form in managing crown rot caused by *S. rolfsii* and can prove as safer and economical method in reducing disease and improving plant stand.

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