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National Academy of Agricultural Science (NAAS) Rating : 3.03

Treatment with Aqueous Leaf Extract of Neem Enhances Seed Germination and Seedling Growth of *Vigna Radiata* L. on Solid Waste Leachate Soil

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ABSTRACT: *Allelopathy is a biological phenomenon by which an organism produces one or more biochemicals that influence the growth, survival and reproduction of other organisms. There may be beneficial (positive) or detrimental (negative) allelopathic effects on the target organisms. Chemicals inhibit the growth of some species at lower concentration may stimulate the growth of other species at higher concentrations. An attempt was done in the present study to evaluate the allelopathic effect of neem extract in the enhancement of seed germination and growth of V. radiata on solid waste leachate soil from Vilappilsala, Thiruvananthapuram. Solid waste leachate soil caused reduction in the germination and seedling growth of V. radiata. Treatment of V. radiata seeds with leaf extract of Azadirachta indica showed enhancement of germination and seedling growth. 1% and 3% of neem extract increased the seed germination upto 100% while higher concentrations inhibited the germination. Treatment of V. radiata with aqueous leaf extract of Neem also increased the length of shoot, root and leaves as well as number of leaves in seedlings grown on solid waste leachate soil. 12 hour treatment with 9% extract induced more than double the number of leaves in solid waste leachate soil grown plants when compared to the untreated plants.*

Key Words: *Allelopathy, Neem, Solid waste leachate soil*

INTRODUCTION

Allelopathy is the direct or indirect stimulatory or inhibitory effects of one plant on another through release of chemical compounds into the environment. Root exudation, leaching by dews and rains, and volatilization or decaying plant tissue from allelopathic plants results in release of compounds into the environment (1). The stimulatory or inhibitory effect of one plant to another depends on the concentration of the released compounds (2). Chemicals inhibit the growth of some species at lower concentration may stimulate the growth of other species at higher concentrations (3). Different groups of plants, crops and weeds have wide known allelopathic interactions (6). Recently, allelopathic studies have become a priority area in weed science researches (7).

Allelopathic crops can be used to control weeds (8) by use of crop cultivars with allelopathic properties, application of residues and straw of allelopathic crops as mulches and use of an allelopathic crop in a rotational sequence where the allelopathic crop can function as a smother crop or where residues are left to interfere with the weed population of the next crop (9, 10). The use of a combination of allelopathy and a strong competitive ability in the crop has been suggested as a beneficial combination for weed management in many crops. The amount of allelochemicals present in a plant is often found to exhibit considerable variation between genotypes and between cultivars. The variation in allelopathic potential between genotypes can be used in the search for crop cultivars with enhanced allelopathic properties (11). If we understand the mechanisms of allelopathic interactions, we can put allelochemicals to work for the benefit of agriculture (12). This objective is of great importance in the design of sustainable agriculture in traditional practices.

The need to reduce harmful environmental effects from the overuse of herbicide has encouraged the development of weed management systems, which are dependent on ecological manipulations rather

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than agrochemicals. Neem is an evergreen tree (*Azadirachta indica)* native to Southeast Asia belonging to the family Meliaceae. Its various parts have been traditionally used to control domestic insects, pests in stored grains, crop pests and in human and livestock medicine. Recently, these properties have been attributed to hundreds of chemicals present in the tree (13). The more common and the most analyzed compounds include nimbin, nimbidin, nimbidol, gedunin, sodium nimbinate, queceretin, salannin, and azadirachtin (14). However, very few reports of neem's allelopathy have been published. With this background, present study has been formulated to evaluate the allelopathic potential of leaf extract of *Azadirachta indica* on seed germination and seedling growth of *Vigna radiata* on solid waste leachate soil from Vilappilsala, Thiruvananthapuram.

MATERIALS AND METHODS

Seed germination and seedling growth of *Vigna radiata* on control and solid waste leachate soil

Healthy seeds of *Vigna radiata* were purchased from the College of Agriculture, Vellayani, Thiruvananthapuram. Fifty seeds of *V. radiata* were sowed in control (normal) as well as in solid waste leachate polluted soil taken from Vilappilsala area. Proper watering was done regularly for germination. The germinated seeds were transferred into separate pots with ten seedlings in each. The seed germination percentage was recorded after three days and morphological observations on length of shoot, root and leaf and number of leaves were recorded after three weeks of germination.

Germination and seedling growth of *Vigna radiate* seeds treated with neem extract

V. radiata seeds were treated with different concentrations of neem (*Azadirachta indica*) leaf extract for various time duration and the treated seeds were tested for the germination and seedling growth in both normal and solid waste leachate polluted soil taken from Vilappilsala area.

Preparation of Neem extract

Green leaves of *A. indica* were picked from the local areas, and then shade dried and powdered. Aqueous leaf extract was prepared by the leaf powder using autoclaved distilled water and kept under laboratory conditions. Concentrations of the prepared extract ranged from 1% to 9% (1%, 3%, 5%, 7% and 9%, w/v).

Seed treatment and Seedling growth test

Seeds of *V. radiata* were dipped in different concentrations of aqueous neem leaf extract (1%, 3%, 5%, 7% and 9%, w/v) for various time duration viz. 2 hours, 4 hours, 6 hours and 12 hours (Table 1). Twenty seeds were used for each treatment. The treated seeds were sowed in solid waste leachate soil taken from Vilappilsala area as well as in normal soil. Control groups were also maintained for both soil types with untreated seeds. Proper watering was done regularly for germination. The germinated seeds were transferred into separate pots with five seedlings in each. Morphological observations were taken for parameters including length of shoot, length of leaves, length of roots and numbers of leaves after three weeks of germination and data were tabulated.

Table 1
Data on seed treatment of Vigna radiata using neem extract

Concentration of neem extract (%)	*Duration of treatment (hrs)*	*Treatment Number*
1	2	T1
1	4	T2
1	6	T3
1	12	T4
3	2	T5
3	4	T6
3	6	T7
3	12	T8
5	2	T9
5	4	T10
5	6	T11
5	12	T12
7	2	T13
7	4	T14
7	6	T15
7	12	T16
9	2	T17
9	4	T18
9	6	T19
9	12	T20

RESULTS

Present study analysed the effect of soild waste leachate soil on seed germination and seedling growth of *Vigna radiata* and also evaluated the allelopathic potential of *Azadirachta indica* leaf extract on enhancing the germination and seedling growth of *V. radiata* on solid waste leachate soil. The control experiments were conducted with untreated seeds on normal soil and solid waste leachate soil which showed notable difference in seed germination percentage. In the control (untreated) experiment, all seeds were germinated in normal soil, but 90% germination was observed in solid waste leachate soil.

Control seeds on solid waste leachate soil showed considerable reduction in germination percentage and shoot length, root length, leaf length and leaf number when compared to those grown in normal soil (Table 2).

Effect of neem extract on seed germination of *V. radiata* in solid waste leachate soil

Seed treatment in *Vigna radaiata* using aqueous neem leaf extract (1%, 3%, 5%, 7% and 9%) done for different time exposures viz., 2hr, 4 hr, 6 hr and 12 hrs showed interesting results (Table 2). Results showed that seed treatment with 1% and 3% neem extract enhanced the germination percentage in solid waste leachate soil, while concentration of neem extract beyond 3% reduced the seed germination percentage to a great extent when compared to that of control (Graphs 1, 2).

Effect of neem extract on shoot length of *Vigna radiata*

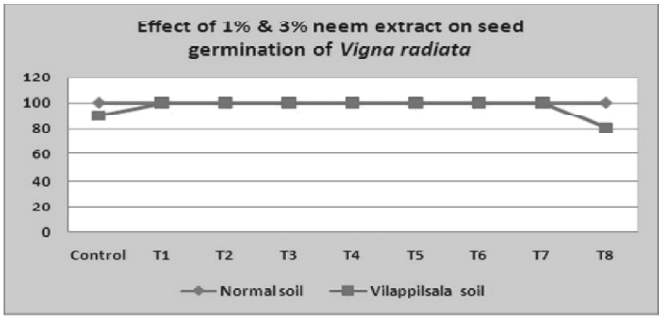
Control plants showed significant difference in shoot length after three weeks of germination with respect to the soil type. Plants in normal soil showed double the length of shoots when compared to that in solid waste leachate soil (Table 2). The shoot length of seedlings germinated on normal soil was almost similar to that of control plants. However, neem extract treatment enhanced the length of shoots in seedlings raised on solid waste leachate soil when compared to that of control (Graph 3). However, the 2 hr treatments were inadequate to increase the shoot length significantly; all the other treatments increased the length of shoots.

Effect of neem extract on root length of *Vigna radiata*

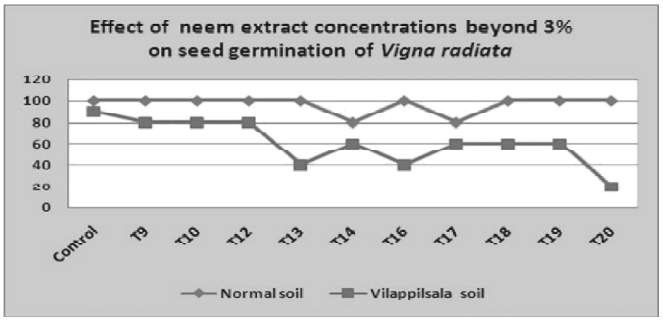
The control seedlings germinated on solid waste leachate soil showed decrease in length of roots when compared to the untreated plants grown in the normal soil. Neem extract treatment increased the length of roots considerably in all the seedlings raised in normal soil. However, in solid waste leachate soil grown plants, the root length was increased significantly in treatments beyond 4 hrs (Graph 4).

Effect of neem extract on leaf length of *Vigna radiata*

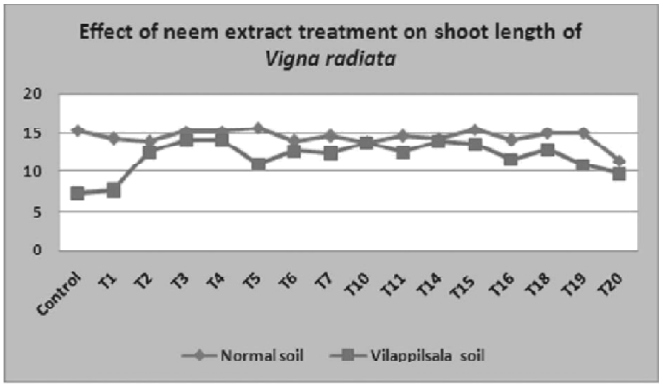
Controls grown on normal and solid waste leachate soils did not showed considerable difference in length of leaves (Table 2). However, neem extract treatment increased the length of leaves in seedlings raised on normal soils except in T12, T15 and T20 (Graph 5). Similarly, seed treatment increased the leaf length in



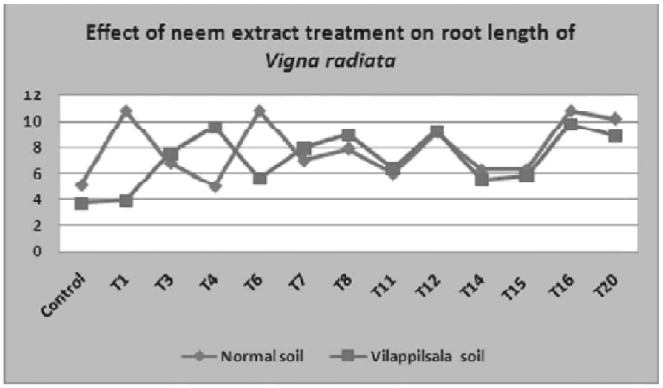
Graph 1



Graph 2



Graph 3

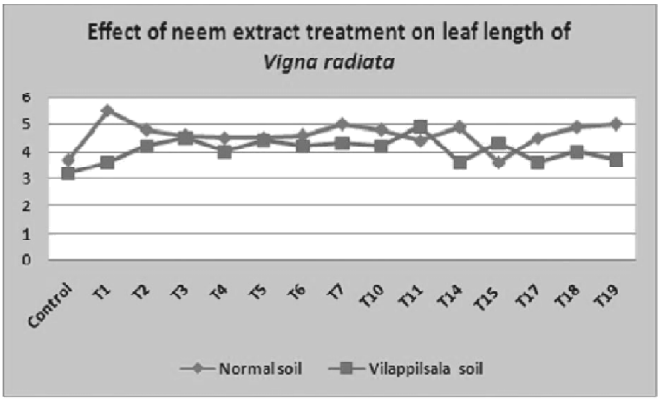


Graph 4

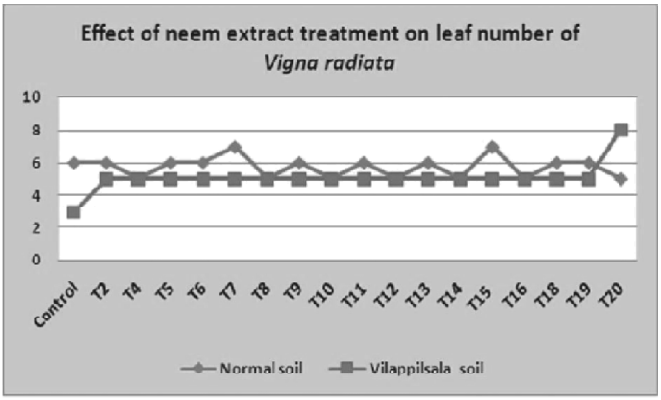
Table 2
Effect of neem extract on seed germination (after 3 days) and seedling growth (after 3 weeks) of *Vigna radiata*

Treatments	*Seed germination (%)*		*Length of shoots (cm) (Mean ± SE)*		*Length of roots (cm) (Mean ± SE)*		*Number of leaves (Mean ± SE)*		*Length of leaves (cm) (Mean ± SE)*	
	Normal soil	*Leachate soil*	*Normal soil*	*Leachate soil*	*Normal soil*	*Leachate soil*	*Normal soil*	*Leachate soil*	*Normal soil*	*Leachate soil*
Control	100	90	15.3 ± 0.12	7.3 ± 0.87	5.1 ± 0.11	3.7 ± 0.74	6.34 ± 0.13	3.01 ± 0.78	3.7 ± 0.54	3.2 ± 0.19
T1	100	100	14.3 ± 0.77	7.7 ± 0.96	10.8 ± 0.34	3.9 ± 0.69	5.97 ± 0.17	2.98 ± 0.34	5.5 ± 0.44	3.6 ± 0.49
T2	100	100	13.9 ± 0.23	12.5 ± 0.12	9.5 ± 0.73	3.2 ± 0.81	6.20 ± 0.46	5.78 ± 0.21	4.8 ± 0.37	4.2 ± 0.44
T3	100	100	15.2 ±0.98	14.0 ± 0.76	6.8 ± 0.91	7.5 ± 0.21	7.01 ± 0.67	3.24 ± 0.55	4.6 ± 0.67	4.5 ± 0.38
T4	100	100	15.2 ±0.12	11.2 ± 0.65	5.0 ± 0.44	9.5 ± 0.29	5.19 ± 0.89	5.98 ± 0.47	4.5 ± 0.71	4.0 ± 0.32
T5	100	100	15.6 ± 0.65	11.0 ± 0.43	9.3 ± 0.39	4.0 ± 0.55	6.98 ± 0.37	5.68 ± 0.76	4.5 ± 0.33	4.4 ± 0.18
T6	100	100	14.0 ± 0.33	12.7 ± 0.39	10.8 ± 0.18	5.6 ± 0.47	6.28 ± 0.29	5.34 ± 0.43	4.6 ± 0.21	4.2 ± 0.31
T7	100	100	14.6 ± 0.45	12.4 ± 0.37	7.0 ± 0.29	7.9 ± 0.54	7.06 ± 0.68	5.45 ± 0.55	5.0 ± 0.37	4.3 ± 0.42
T8	100	80	15.5 ± 0.97	9.5 ± 0.42	7.9 ± 0.74	8.9 ± 0.91	5.45 ± 0.78	5.89 ± 0.78	3.7 ± 0.68	3.3 ± 0.81
T9	100	80	15.0 ± 0.21	7.2 ± 0.57	8.8 ± 0.64	3.6 ± 0.26	6.81 ± 0.39	5.76 ± 0.32	4.8 ±0.43	2.4 ± 0.79
T10	100	80	13.8 ± 0.34	13.7 ± 0.86	6.0 ± 0.66	4.9 ± 0.13	5.26 ± 0.25	5.23 ± 0.57	4.8 ± 0.42	4.2 ± 0.37
T11	100	100	14.6 ± 0.76	12.5 ± 0.22	6.0 ± 0.93	6.4 ± 0.16	6.31 ± 0.34	5.29 ± 0.34	4.4 ± 0.19	4.9 ± 0.15
T12	100	80	13.6 ± 0.45	8.6 ± 0.84	9.1 ± 0. 16	9.2 ± 0.19	5.79 ± 0.17	5.87 ± 0.42	3.2 ± 0.28	2.8 ± 0.16
T13	100	40	14.1 ± 0.43	7.5 ± 0.29	7.0 ± 0.19	2.6 ± 0.82	6.78 ± 0.19	5.11 ± 0.14	4.2 ± 0.17	3.3 ± 0.49
T14	80	60	14.2 ± 0.11	13.8 ± 0.30	6.3 ± 0.12	5.5 ± 0.76	5.31 ± 0.67	5.34 ± 0.32	4.9 ± 0.16	3.6 ± 0.40
T15	100	100	15.4 ± 0.14	13.5 ± 0.21	6.3 ± 0.29	5.8 ± 0.33	7.49 ± 0.64	5.25 ± 0.33	3.6 ± 0.52	4.3 ± 0.31
T16	100	40	14.1 ± 0.56	11.5 ± 0.19	10.8 ± 0.31	9.7 ± 0.38	5.29 ± 0.53	5.75 ± 0.29	4.8 ± 0.31	2.7 ± 0.36
T17	80	60	13.7 ± 0.65	7.5 ± 0.81	9.8 ± 0.30	2.5 ± 0.27	6.23 ± 0.52	2.12 ± 0.54	4.5 ±0.39	3.6 ± 0.15
T18	100	60	15.0 ± 0.55	12.8 ± 0.21	6.6 ± 0.20	3.9 ± 0.59	6.45 ± 0.24	5.12 ± 0.86	4.9 ± 0.39	4.0 ± 0.12
T19	100	60	15.0 ± 0.27	10.8 ± 0.34	6.6 ± 0.29	4.5 ± 0.64	6.76 ± 0.52	5.27 ± 0.49	5.0 ±0.19	3.7 ± 0.61
T20	100	20	11.4 ± 0.73	9.6 ± 0.33	10.2 ± 0.18	8.8 ± 0.55	5.81 ± 0.29	8.61 ± 0.44	3.6 ± 0.16	2.1 ± 0.49.

Mean ± SE were calculated from twenty samples



Graph 5



Graph 6

solid waste leachate soil grown plants also, though, T9, T12, T16 and T20 showed reduced leaf length when compared to the control.

Effect of neem extract on leaf number of *Vigna radiata*

Controls raised on normal and solid waste leachate soil showed significant difference in the number of leaves. The later exhibited only half the number of leaves when compared to that of former. Seed treatment did not exerted any significant effect in normal soil grown plants, although a marginal increase was noticed. Whereas, neem extract showed a profound effect on solid waste leachate soil grown plants by increasing the number of leaves significantly (Graph 6). The T20 treatment induced more than double the number of leaves in solid waste leachate soil grown plants when compared to that of control (Table 2).

DISCUSSION

Azadirachta indica A. Juss (Neem) is well known in India for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. It has been extensively used in ayurveda, unani and homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of neem. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. The Neem extract has undergone extensive pharmacological screening and found to have several pharmacological activities due to the presence several active constituents in it (Biswas 15). Present study exploited the efficacy of aqueous leaf extract of neem to enhance seed germination and seedling growth of *Vigna radiata* on solid waste leachate soil.

Solid Waste is the term used to describe non-liquid waste material arising from domestic, trade, commercial, agricultural, industrial activities, and from public services

Unscientific and crude method of landfill, 'open dumping' is the main method of waste disposal prevailing in the major cities of Kerala. It is common because it is cheap & some waste cannot be treated otherwise. As water percolates through the waste site, contaminants are leached from the solid waste, and a complex sequence of physical, chemical, and biological mediated events leads to the production of solid waste leachate. Leachate migration from wastes sites and the release of pollutants from sediments pose a high risk to groundwater resource if not adequately managed. Toxic chemicals that have high concentration of nitrate and phosphate derived from waste in the soil can filter through a dump and contaminate both ground and surface water.

The centralized solid waste management plant located in the outskirts of the Thiruvananthapuram City (16kms away from city center) at Vilappilsala manages the recycling of garbage generated in the City. But solid wastes are being dumped in the area due to the reduced recycling capacity of the plant making both land and water resources severely

polluted. Most of the solid wastes, like paper, plastic containers, bottles, cans and electronic goods are not biodegradable and they do not get broken down through inorganic or organic processes. Thus, when they accumulate they pose a health threat to people. Moreover, it also causes damage to terrestrial organisms and reduces the use of land for other more useful purposes. The dumping of waste has been started in the Vilappilsala area before ten years and the solid waste leachate caused major problems in the adjoining areas including many diseases and health hazards. It has also resulted in the pollution of Karamana River and become a major threat to the nearby population.

Present study analysed the effect of soild waste leachate soil on the germination and seedling growth of *Vigna radiata*. The control experiments were conducted with untreated seeds on normal soil and solid waste leachate soil and showed significant difference on seed germination percentage and morphological parameters with respect to the soil type. Only 90% of *V. radiata* seeds were germinated in the solid waste leachate soil while 100% germination was observed in the normal soil. Seed germination and growth are vital for continuation of life of seeds. Seedlings are extremely vulnerable to environmental stresses due to presence of polluting agents in the environment especially during seed hydration period which is very important for irrigation and triggering the intricate sequences of metabolism essential for germination and growth of seedlings. The effect of industrial effluents on growth and yield parameters of agricultural crops and soil properties has been extensively studied (16, 17). But only few studies are made to find out the effect of industrial effluents on germination (18, 19).

Rubber factory effluents reduced the percentage of seed germination in *V. radiata* (20) similar to present results

Present study also observed the arrest of seedling growth in solid waste leachate soil. The length of shoots, roots, leaves and number of leaves showed significant reduction in seedlings grown on the solid waste leachate soil when compared to those in normal soil. Suppressed growth of cowpea seedlings in more than 10% concentration of distillery effluents has been reported earlier (21). Distillery effluent had serious deleterious effects in the early seedling growth of pigeon pea also (22).

In the present study, seed treatment with aqueous leaf extract of *A. indica* increased the percentage of seed germination up to 100%. The lower concentrations (1% & 3%) of neem extract were effective for increasing the seed germination percentage in solid waste leachate soil, while, increase in the concentration of the extract beyond 3% decreased the germination percentage. Only 20% germination was observed in seeds treated with 9% neem extract for 9 hours. Inhibitory effect of higher concentrations of neem extract on seed germination has been reported in a wide range of species (23). Treatment with neem extract in the present study enhanced the growth of seedlings on solid waste leachate soil. Neem extract treatment increased the length of shoots, roots and leaves in these seedlings along with induction of more number of leaves. The seed quality characters of neem with industrial effluents viz., tannin, textile dyeing, cement, rayon pulp and automobile both as raw and in different dilutions have been evaluated (24) and revealed that on irrigation with raw material, the reduction in germination was minimal due to tannin and rayon pulp. In the present study, neem leaf extract was effective for increasing the seed germination and growth of *V. radiata* on solid waste effluent soil. Results indicate that neem leaf extract has a significant effect on enhacing seed germination and seedling growth on solid waste leachate soil which can be used as an efficient alternative way for the revitalization of solid waste dumped areas.

ACKNOWLEDGEMENT

First author is thankful to University Grants Commission, New Delhi for the award of UGC Research Award 2012-'14.

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