EFFECT OF DIFFUSATES OF *PARTHENIUM HYSTEROPHOROUS* L. ON SEED GERMINATION OF *RAPHANUS SATIVUS* L.

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Abstract: The present investigation focused on the effects of plant and seed diffusates of *Parthenium hysterophorus* L. on seed germination of *Raphanus sativus* L. Level of glucose in germinating seeds as an indirect measure of amylase activity was estimated. About 91.66% viable seed of *Raphanus sativus* were treated in different concentrations of rhizospheric soil diffusates (PD) and germinating seed diffusates (SD) of *Parthenium hysterophorus*. Both diffusates were found effective to retard and minimize the germination of *Raphanus* seeds. The maximum inhibition for both cases was found at 100 % concentrations of the diffusates. The correlation analysis revealed the correlation factor - 0.784 between PD concentrations and *Raphanus* seed germination and that of - 0.889 between SD concentrations and *Raphanus* seed germination with 5% level of significance. This indicated that the increase in concentrations played negative role in germination process of *Raphanus* seeds. The results were interpreted in terms of the possible roles of "Parthenin", a sesquiterpene-lactone which is known to be present in *Parthenium hysterophorus*.

Key words: Sesquiterpenes; Allelopathy; Parthenin.

INTRODUCTION

Parthenium hysterophorus L., a member of Asteraceaea family is a noxious herbaceous weed has infested both fallow and cultivated lands in tropical Asia including Nepal. Its occurrence was first reported in Nepal in 1982 by *Hara et al*. By then it was of sporadic occurrence but now it is most common. It has prolific seed producing ability (Haseler, 1976) and the plants have significant allelopathic impacts on neighboring plant species (Adkins and Sowrby, 1996). It dominates field crops as competitive weed (Tamado *et al* 2002). Regular contact with the plant or its pollen may cause dermatitis, hay fever, and asthma to human beings (Anonymous, 2004). It is also reported to have adverse effects to livestock and cattle and even can cause death if consumed in significant amount (CRC, 2003). A comprehensive account on its phenology has been reported in the pest series publication of Queensland government and the Natural heritage trust, Australia (Anonymous, 2004; CRC, 2003).

An allelopathic influence of *Parthenium hysterophorus* on the early growth of *Brassica* species by releasing water soluble phenolics into the soil (Batish *et al*., 2005), seed germination and seedling growth of *Eragrostis tef* (Tefera, 2002), sorghum yield loss by the weed density and duration of competition, peaking at 97 % (Tamado *et al.*, 2002) also have been reported. The plant contains 'Parthenin', an active chemical which is a terpenoid (Sesquiterpene). This group of chemicals is reported to affect the early growth and physiology of *Ageratum conizoides* (Batish *et al*., 2002), *Triticum aestivum* (Patil & Hedge, 1988) and even 90 % pollen sterility in *Raphanus sativus* (Bhatt *et al.,* 1994).

The glucose content in seeds treated with allelochemicals with source in plant or seed diffusates may be taken as an indirect measure of the activity of hydrolytic enzymes that are active during germination to supply glucose to glycolytic pathway of respiration. The present investigation involves this principle targeting to analyze the allelochemic effects of plant and seed diffusates of *Parthenium hysterophorus* L. on *Raphanus* seed germination.

MATERIALS AND METHODS

Source of seeds

Seeds of *Parthenium hysterophorus* L. were collected from Kirtipur during August and dried in shade for about a week. Air dried seeds with moisture content below 12%, as managed through repeated weighing and drying, was taken for use in the experiments. Seeds of Radish (*Raphanus sativus* L. var. White Neck), belonging to previous harvest, was obtained from the Kalimati vegetable market in Kathmandu. The seeds used in experiments were manually selected for similarity and healthiness.

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Diffusates

Plant Diffusates (PD):

Adhering soil mass to the roots of matured and flowering *Parthenium* plants was taken as the source plant diffusates. Soil samples were collected from the roots of freshly uprooted plants in the evening of a hot sunny day with no rain-fall in the week. Known quantity (10 g.) of this rhizospheric soil samples were then steeped in 100 ml distilled water for 24 h. at room temperature (25^{0}C). Double filtration was carried out; the first through clean muslin cloth and the second using Whatman No.1filter paper. The filtrates (85 ml approx) was considered as the 100% PD and used as stock.

Seed Diffusates:

Seed diffusates (SD) was obtained from plain agar (2 %) plate planted with *Parthenium* seeds. Healthy seeds were manually selected for use in the experiments. Well imbibed seeds (36 h in distilled water at room temperature) were planted into plates containing plane agar (2.5 %) in batches of 50 seeds per plate at equidistance. Plates were kept into incubator (25 ºC) for a week. Moisture (2 ml sterilized distilled water per plate) was added at every 4th day until most of the seeds germinated. At 90 % germination (approx) the seedlings of *Parthenium* were removed from the plates. The agar mass were then taken out from all plates and placed into a beaker (100 ml) containing 50 ml sterilized distilled water and mixed thoroughly. After a period of 24 h the slurry was well stirred and centrifuged. The supernatant was used as the source of seed diffusates (SD) containing allelopathic chemicals. It was used as 100 % SD stock.

Experiments

Both the diffusates were then diluted to required concentrations (20 – 100 %) by dilution for use in experiments.

Germination Studies:

Sixty seeds of *Raphanus sativus* were soaked overnight in different concentrations of plant diffusates (PD) and seed diffusates (SD). They were sown in Petri dishes at equidistance on a single layer of filter paper moistened (1.5 ml) with corresponding concentrations of PD or SD. Each of the experimental unit was replicated thrice. A control group was made by soaking the seeds in distilled water.

The plates were incubated (25 ºC in dark) for 12 h with hourly observation. The seed germination data was then taken after a constant value reached by the control set.

Glucose estimation

Concentration of glucose in germinated seeds belonging to different experimental unit was estimated using Sumogyi and Nelson colorimetric method (1951). The principle of this method is the reduction of alkaline copper reagent by glucose as indicated by blue coloration of the test solution. The level of glucose was estimated by measuring the absorbance value of test solutions (Extracted from germinating seeds) using pure glucose solution as internal standard at 560 nm. The value of glucose content and percent reduction in its level was calculated using the formula:

a) $$\text{Glucose prod. / Seed} = \frac{A_{560} \text{ of test sol}^{n.} \text{ X Std. glucose conc. /unit } A\text{-}_{560}}{\text{No. of seeds}} \text{ X dil. Factor*}$$

b) $$\text{\% Reduction of glucose / Seed} = 100 - \frac{\text{Glu. Content / seed on respective media}}{\text{Glu. Content / seed in D/W media}} \text{ X100}$$

* The value of dilution factor in the equation is 500 since the extracts were diluted 500 times.

RESULTS AND DISCUSSION

Germination Studies:

The present investigation analyzed the allelochemic effect of plant and seed diffusates of *Parthenium hysterophorus* L. on seed germination of Radish (*Raphanus* sativus L. var. White Neck). The *Raphanus* seeds are one of the fast germinating seeds and were found to germinate within 10-12 h. at 25° C in laboratory. Germination of *Raphanus* seeds followed a sigmoidal germination pattern in control condition with the maximum germination yield (91.66%) in between 5-10 h as monitored on an hourly basis. Because of its photoblastic nature the seeds were kept in dark upto the emmergence of radicles (Gaurdia *et al*., 1987). In principle the imbibed seeds need glucose molecules to gear up the glycolytic respiratory process to support the germination to proceed.

Fig. 1. shows the low germination of *Raphanus* seeds in PD reaching to no germination at 100 % concentration in comparision to the SD. The correlation analysis revealed the correlation factor – 0.784 for PD and - 0.889 for SD ($P < 0.05$). This indicated the presence of germination inhibitor in the diffusates. Although differed for magnitude both PD (the rhizospheric plant diffusates) and SD (seed diffusates) inhibited the seed germination.

The plant contains 'Parthenin', an active chemical which is a terpenoid (Sesquiterpene). This group of chemicals is reported

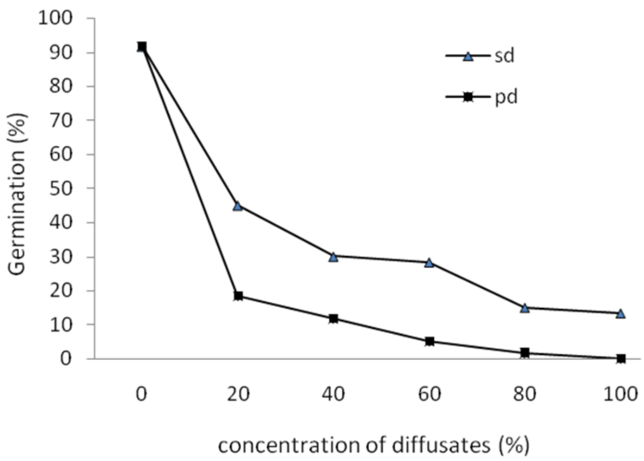

Fig. 1: Germination of *Raphanus* seeds at different concentrations of plant diffusates (PD) and seed diffusates (SD). Germination temperature was 25 ^{0} C.

to affect the early growth and physiology of *Ageratum conizoides* (Batish *et al*., 2002), *Triticum aestivum* (Patil & Hedge, 1988) and even 90 % pollen sterility in *Raphanus sativus* (Bhatt *et al.,* 1994). The allelopathic influence of *Parthenium hysterophorus* on the early growth of *Brassica* species was found linked to the release of certain water soluble phenolics into the soil (Batish *et al*., 2005).

Data were consistent for increasing concentrations affected seed germination negatively for both PD and SD. The maximum inhibition for both was found at 100 %.

The low germination of *Raphanus* seeds in the presence of seed and plant diffusates of *Parthenium* is probably due to the effect of the chemical parthenin, a sesquiterpene - lactone which is likely to be present in the plant and obviously in its diffusates. The chemical is responsible for strong inhibitory influence of the weed to many neighbouring plants (Adkins and sowerby, 1996) and strong competitiveness with crop plants (Tamado *et al*., 2002). Among several biologically active compounds, the phytotoxic potential of sesquiterpene has long been established (Dayan *et al.*, 1999). Furthermore, an allelopathic influence of water soluble phenolics released by the plant into the soil is reported on the early growth of *Brassica* crops (Batish *et al*., 2005) and seed germination and seedling growth of *Eragrostis tef* (Tefera, 2002). Recently, Regina *et al.* (2007) investigated the level of involvement of Parthenin in overall phytotoxicity of decomposing leaf material in a South African population of *P. hysterophorus*. Results showed that the contribution of Parthenin is highly dependent on its concentration within extract solutions and varied between 16% and 100% of overall phytotoxicity of leaf extracts. Besides, Parthenin treatments are proven to delay germination and stimulate root growth at low doses in various experiments.

***Glucose Content in germinating seeds*:**

The glucose content in germinating seeds, treated with allelochemicals with source in plant or seed diffusates, was taken as an indirect measure of amylase activity linked to the supply of glucose to respiratory process of germinating seeds. The treated seeds (PD and SD) were compared with control for the content of glucose which in tern taken as an indirect measure of amylase activity. Reference value for Standard Glucose was also obtained and ploted for comparisons (Fig.2.) The glucose content in both PD and SD treated seeds were found decreased in comparision to that of the distilled water (control). The relative concentration of glucose (OD at 560nm) was found decreased by 55% and 37% corresponding to PD and SD treaments respectively ($P < 0.05$). Hence both of the diffusates inhibited amylase activity with the PD treatment more effective than SD as measured on the basis of glucose content in the germinating radish seeds.

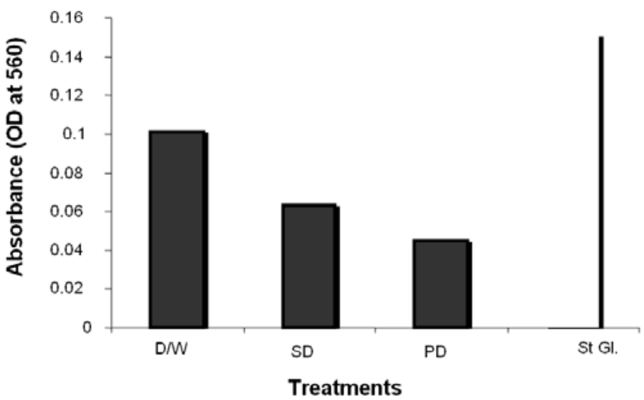

Fig: 2: OD at 560 nm (average of four independent samples) for different treatments : DW= Distilled Water; SD= Seed Diffusate ; PD= Plant Diffusate : St. Gl = Standard Glucose.

Various reports suggest high concentration of parthenin in the root of the plant (Kanchan and Jayachandra 1979, 1980 a). Furthermore, leaf residues are believed to deliver large amounts of Parthenin to soil during decomposition (Regina *et al*., 2007). Obviously the accumulation of parthenin and probably the other associated chemicals were at high conentration on the soil.

CONCLUSSION

From the overall experiments it can be concluded that the plant released and seed released diffusates from invasive alien weed *Parthenium hysterophorus* had allelopathic impact on the *Raphanus sativus* in laboratory conditions. Increase in concentrations of both diffusates decrease Raphanus seed germination. The diffusates not only inhibits the germination of *Raphanus* seeds but minimize the glucose level produced during their germination too.

Thus the high effectiveness of the plant diffusates in inhibiting the *Raphanus* seed germination due to high concentration of the parthenin is possible.

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