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Performance of *Vicia faba* Plants in Relation to Simulated Acid Rain and/or Endosulphan Treatment

Nandita Singh, Mohd. Yunus, S. N. Singh, and K. J. Ahmad

Environmental Botany Laboratory, National Botanical Research Institute, Lucknow-226 001, India

Acid rain is largely derived from oxides of sulphur and nitrogen that emanate from fossil fuel combustion. In eastern North America and several countries of Europe, acid rain is considered to be a factor in 'wildstraben'/'forest die back'. Various deleterious effects (direct and indirect) of acid rain on vegetation have been extensively reviewed by Evans (1988). In India too, areas around industrial agglomerations and urban areas burdened with motorized vehicular traffic are no longer pristine. In Bombay, Trombay and Chembur areas have already experienced acidic precipitation.

The increasing human population in India is necessitating the optimum use of cultivable land for increased food production. Eradication and control of pests and pathogens is an essential component of any strategy for increased agricultural production. In this context, the use of pesticides to control the incidence of disease in crops becomes inevitable. Most of the pesticides used for foliar spraying invariably contain surfactants in their formulation, which not only increase the surface wettability but also enhance permeability of the cuticle for more cation infusion/effusion and hence they may make the leaf more susceptible to direct effects of acid rain (Kirkwood, 1987). To evaluate the validity of this assumption, an experiment using endosulphan, the most commonly used insecticide in India, and acid rain of different pH was conducted on *Vicia faba*.

MATERIALS AND METHODS

Five seeds of *Vicia faba* L. var. Seo-chana were sown in each 30 cm earthen pot, lined with polyethylene containing a 1:1 mixture of garden soil and compost manure. An equal amount (2 g) of N-P-K mixture was added to each pot to improve the nutrient status. Plants were watered on as needed but avoiding foliar washing. Before treatment application plants were thinned to one plant per pot of comparable growth and vigour.

A 0.2% solution of endosulphan (thiodan, commercial formulation Hoechest of 95-98% purity) was used in this experiment. Acid rain of three different pH (5.6, 4.0 and 2.8) were prepared using deionized water, sulphuric and nitric acids of a sulphate (SO_4^{2-}) to nitrate (NO_3^{-})

Send reprint requests to Nandita Singh at the above address.

ratio of 2:1 and appropriate concentration (ppm) of cations and anions (Na-8.4, K-1.2, Ca-17.4, Fe-0.28, Cu-0.02, Zn-0.14, Pb-1.06, Cl-0.10, PO_4-0.26, NH_4-0.26).

A hand stainless steel sprayer was used to spray endosulphan or/and acid rain at the rate of 6.7 mm h^{-1}. Five ml of endosulphan or/and acid rain per plant per treatment were sprayed.

Plants were grouped into five sets for the following treatments:

- Standard control- plants, sprayed with deionized water.
- Plants sprayed with simulated acid rain of three different pH (5.6, 4.0, 2.8).
- Plants sprayed with 0.2% endosulphan (pH 6.5).
- Plants sprayed with endosulphan followed by acid rain.
- Plants sprayed with acid rain followed by endosulphan.

Experiments had five replications and were repeated twice. Sampling was done when the plants age was 40 (Pre-flowering), 60 (Flowering) and 80 days (Post-flowering).

Plants were examined regularly to observe the emergence of any symptom/injury. On each sampling day plants were excavated, washed, partitioned into root and shoot and dried for their constant weights for phytomass determination. Photosynthetic pigments and protein were estimated following the methods of Lichtenthaler and Wellburn (1983) and Lowry *et al.* (1951), respectively.

Statistical analysis (analysis of variance and Duncan's multiple range test) were performed on all experimental data.

RESULTS AND DISCUSSION

The study revealed that the sequence of treatments application did influence the response of plants.

Plants treated with acid rain of pH 2.8, showed foliar phytotoxic symptoms mostly on leaf margins and interveinal areas. Premature senescence of the affected leaves was also witnessed. Plants treated with endosulphan and subsequently with acid rain of pH 2.8 showed more foliar injury than those exposed to pH 2.8 alone or acid rain of pH 2.8 followed by endosulphan. Foliar size was also considerably reduced in the treated plants (Table 1). Plants exposed to acid rain of higher pH (4.0 and 5.6) with or without pesticide, did not manifest any visible injury symptoms.

In all treatment combinations, except in acid rain of pH 5.6, reduction of chlorophyll level in plants treated with endosulphan followed by acid rain was more than in plants exposed to either acid rain of pH 2.8 or endosulphan singly or acid rain followed by endosulphan (Table 1).

The protein (a measure of crop quality) concentration also decreased in all treatment combinations in comparison to control and the trend of change was the same as in the chlorophyll (Table 2).

Acid rain and endosulphan both had an adverse impact on the growth and development of the plants. The root and shoot lengths and number

Table 1. Leaf area (cm^2 $plant^{-1}$) and total chlorophyll (mg g^{-1}fr.wt.)of *Vicia faba* treated with endosulphan and simulated rain of different pH at flowering stage (values are mean from five observations).

Treatment sequence	Leaf area	Total chlorophyll
0 AR + 0 E	410.0 *b*	15.6 *bc*
0 AR + 0.2 E	399.3 *c* (-2.6)	15.8 *b* (1.3)
5.6 AR + 0 E	418.9 *a* (2.2)	17.0 *a* (8.9)
5.6 AR + 0.2 E	377.2 *d* (-8.0)	15.2 *c* (-2.6)
4.0 AR + 0 E	337.3 *e* (-17.7)	14.3 *e* (-8.4)
4.0 AR + 0.2 E	321.8 *f* (-21.5)	12.6 *f* (-19.2)
2.8 AR + 0 E	305.5 *g* (-25.5)	11.5 *g* (-26.3)
2.8 AR + 0.2 E	210.6 *h* (-48.6)	10.5 *gh* (-29.5)
0.2 E + 5.6 AR	409.6 *b* (-0.1)	15.0 *d* (-3.8)
0.2 E + 4.0 AR	323.2 *f* (-21.2)	10.3 *h* (-33.9)
0.2 E + 2.8 AR	183.6 *i* (-55.2)	8.1 *i* (-48.1)

AR= Acid rain (pH); E= Endosulphan (%)

- Means in column followed by the same superscript are not significantly different at the 5% level according to Duncan Multiple range test.
- Values in parenthesis indicate per cent change from control.

of nodules decreased significantly over the control (Table 3). On the other hand, the plants treated with pH 5.6 acid rain, showed better growth.

The phytomass which is the ultimate product of metabolic activities, increased in all sets of experiments till maturity, but decreased during the senescence (Table 2). Though pesticide alone did not affect the plant growth much, it caused significant decrease in the phytomass accumulation. This was particularly evident when the pesticide was sprayed prior to acid rain treatment.

Results reported herein show that *Vicia faba* is very sensitive to acid rain. Its sensitivity increases when acid rain is combined with endosulphan.

Table 2. Protein content (mg g^{-1}fresh wt of leaf) at flowering stage and phytomass (g dry wt. $plant^{-1}$) at post flowering stage of *Vicia faba* treated with endosulphan and simulated rain of different pH (values are mean from five observations)

Treatment sequence	Protein content	Phytomass
0 AR + 0 E	36.7 *b*	123.6 *ab*
0 AR + 0.2 E	37.4 *a* (1.9)	120.7 *abc* (-2.3)
5.6 AR + 0 E	37.9 *a* (3.3)	124.9 *a* (1.0)
5.6 AR + 0.2 E	34.6 *c* (-5.7)	121.8 *abc* (-1.4)
4.0 AR + 0 E	30.2 *d* (-17.7)	117.1 *bcd* (-5.2)
4.0 AR + 0.2 E	26.1 *e* (-29.7)	112.6 *d* (-8.8)
2.8 AR + 0 E	25.8 *f* (-29.7)	104.0 *e* (-15.8)
2.8 AR + 0.2 E	21.3 *g* (-41.9)	101.0 *e* (-18.2)
0.2 E + 5.6 AR	34.0 *c* (-7.3)	116.5 *bcd* (-5.7)
0.2 E + 4.0 AR	22.6 *g* (-38.4)	97.8 *f* (-20.8)
0.2 E + 2.8 AR	15.2 *h* (-58.6)	64.8 *g* (-47.5)

AR= Acid rain (pH); E= Endosulphan (%)

- Means in column followed by the same superscripts are not significantly different at the 5% level according to Duncan multiple range test.
- Figures in parenthesis indicate % change from control.

The dorsiventrally flattened and pubescent (hairy) foliar surface of *V. faba* does not retain rain drops for long periods because of the angle of contact between the drops and the foliar surface (Martin and Juniper 1970). It has been observed that endosulphan affects the submicroscopic structure of the epicuticular wax layer, consequently increasing the surface wettability and enhancing the penetration of endosulphan and hydrogen ions.

Acidic precipitation may have adverse effects on plant growth and productivity (Evans *et al.* 1982). Dry weights and lengths of root and shoot and the leaf area of acid rain treated plants were reduced by high rain acidity (pH 2.8). The combined treatment of acid rain and endosulphan showed an enhanced effect on plant growth, as endosulphan

Table 3. Response of *Vicia faba* plants treated with endosulphan and simulated rain of different pH at flowering stage (values as mean from five observations)

Treatment sequence	Root length (cm)	Shoot length (m)	Nodule number
0 AR + 0 E	18.6 *b*	57.7 *a*	24 *b*
0 AR + 0.2 E	17.1 *d* (-8.4)	52.1 *abc* (-9.8)	22 *c* (-8.3)
5.6 AR + 0 E	19.3 *a* (2.4)	52.6 *ab* (-8.8)	25 *a* (4.1)
5.6 AR + 0.2 E	18.0 *c* (-3.4)	48.2 *bcd* (-16.4)	21 *d* (-12.5)
4.0 AR + 0 E	16.2 *c* (-12.8)	48.8 *bcd* (-15.5)	20 *e* (-16.6)
4.0 AR + 0.2 E	14.2 *fg* (-23.9)	45.6 *cd* (-21.0)	18 *f* (-25.0)
2.8 AR + 0 E	14.4 *f* (-22.4)	44.3 *de* (-23.2)	14 *g* (-41.6)
2.8 AR + 0.2 E	12.0 *gh* (-35.5)	38.4 *e* (-33.4)	12 *h* (-50.0)
0.2 E + 5.6 AR	13.5 *g* (-27.6)	44.1 *de* (-23.6)	20 *e* (-16.6)
0.2 E + 4.0 AR	11.9 *h* (-36.1)	38.0 *e* (-34.2)	14 *g* (-41.6)
0.2 E + 2.8 AR	9.5 *i* (-48.7)	29.1 *f* (-49.5)	10 *i* (-58.3)

AR= Acid rain; E= Endosulphan.

- Means in column followed by the same superscripts are not significantly different at the 5% level according to Duncan Multiple range test.
- Values in parenthesis indicate % change from control.

would have also caused a decrease in levels of cellulose and hemicellulose, by blocking their synthesis, and levels of pectins by accelerating their degradation, as reported earlier by Agarwal and Beg (1982). Interference of endosulphan in mobilization of food reserves and their subsequent utilization in the developing plant parts could have also affected the plant growth adversely. Such effects of pesticides were observed by Dalvi and Salunkhe (1975).

Reduction in chlorophyll concentration increased with rain acidity (Fernbaugh 1976, Hindawi *et al.* 1980) but the effect was more pronounced at pH 2.8 than at pH 4.0 and 5.6. Perhaps higher rain acidity either retarded chlorophyll synthesis or induced the breakdown of chlophyll molecules. Further, the presence of endosulphan with the acid rain treatment stimulated chlorophyll reduction.

It may be concluded that the sensitivity of plants to acid rain (pH 2.8 and 4.0) was significantly increased by prior exposure to endosulphan. This synergism between acid rain and endosulphan could be due to increased ionic imbalance of the plant leaves as the pesticide (foliar sprayed one) changes the foliar surface characteristics by disturbing the epidermis, cuticle and wax layers (Mersie and Foy, 1986) thus facilitating the infusion of endosulphan molecules from leaf surface and also hydrogen ions. The synergism could also involve an influence of low pH on the degree of dissociation of the pesticide molecule which might enhance its penetration through the cuticle.

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