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EFFECT OF HIGH CALCIUM AND PHOSPHORUS ON THE GROWTH AND DEVELOPMENT OF *METOPOLOPHIUM DIRHODUM* AND ITS PARASITOID *APHIDIUS RHOPALOSIPHI*

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ABSTRACT: Glasshouse studies were conducted to ascertain the effects of nutrient solutions of calcium and phosphorus on the growth and development of rose grain aphid, *Metopolophium dirhodum* (Walker) and its parasitoid *Aphidius rhopalosiphi* (De Steph.). High calcium and high phosphorus concentrations applied to a partially resistant wheat cultivar "Rapier" did not have a significant effect on the growth and development of the aphid or its parasitoid. However, the mean fecundity of the parasitoid was higher on plants with high phosphorus than those treated with high calcium concentrations.

Key Words: *Metopolophium dirhodum; Aphidius rhopalosiphi; Calcium; Phosphorus; Development; Fecundity; Pakistan.*

INTRODUCTION

It is well documented that cereal plant growth is enhanced with increased application of nitrogen. An adequate supply of potassium and phosphorus increases the hardiness of the plants, making them less subject to lodging. Phosphorus is an essential component of living matter. Second to nitrogen it is the most limiting element in soils (Yoshida, 1981). It is one of the prime examples of interactions with other elements especially the micronutrients (Clark, 1982). Phosphorus deficiency inhibits protein metabolism which results into soluble nitrogen (Eaton, 1952). This may cause a decrease in sugars and auxins but an increase in osmotic pressure and plasma viscosity (Pirson, 1955). The effects of these nutrients on aphids' development have been little investigated but available evidence has shown an increased infestation by *Myzus persicae* and other aphid species on plants that were given low or no phosphorus.

Calcium is required in large amounts by plants for cell division, cell elongation and the detoxification of H+ ions and increases in nitrogen availability (Burstrom, 1954; Pirson, 1955). It increases potassium uptake by roots (Nielsen and Overstreet, 1955) but appears to have an antagonistic effect on potassium and manganese ions in enzymatic reactions (Kachmar and Boyer, 1953). Calcium and magnesium concentration in plant shoots increased with increased calcium and magnesium in soil (Favaretto et al., 2008). Increasing soil calcium level may increase NH_4 concentration in soil solution (Fenn and Feagley, 1999) by competition for soil exchange sites, and this can interfere with nutrient availability and plant growth (Stout et al., 2003; Fenn and Feagley, 1999). EI-Tigani (1962) reported that calcium deficient plants are vulnerable to aphid attack. With calcium deficiency, larger amount of manganese are taken up by plants and manganese toxicity may result. Effect on growth and development of aphid by plant nutrition (fertilisers or chemicals responsible for natural resistance in plants), will in turn affect the size and development of the parasitoid. Consequently, the effect of nutritional stress at the first trophic level is passed on to the tertiary trophic levels.

Various workers also reported positive interactions on tritrophic levels involving the partially resistant wheat cultivar 'Rapier' fertilised with high nitrogen and potassium nutrient solutions. Present work aimed to investigate whether such an interaction occurs when the "Rapier" is fertilised with other nutrients such as high calcium and phosphorus.

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MATERIALS AND METHODS

Seeds of the partially resistant wheat cultivar 'Rapier' were sown in John Innes No. 2 compost in 9 cm diam. plastic pots. The nutrient solutions were prepared in distilled water from stock solutions (based on Hewitt, 1952) (Table 1). Ten days after germination, when the seedlings were at two leaf stage plants were thinned to four per pot and 50 ml of nutrient solution was applied to wheat plants once a week. All the experiments were organised as a Completely Randomised Design.

The treatments were:

1) High Calcium: Low phosphorus
   $CaCl_2$ $6H_2O$ = 44.00 $ml l^{-1}$
   NaH_2 $P0_4$ $2H_20$ = 5.00 $ml l^{-1}$
2) High Phosphorus: Low calcium
   NaH_2 $P0_4$ $2H_20$ = 12.50 $ml l^{-1}$
   $CaCl_2$ $6H_2O$ = 5.00 $ml l^{-1}$

All other salts and micronutrients remained constant.

A culture of *M. dirhodum* was established on the wheat cultivar Rapier inside muslin cages (45cm x 45cm x 45 cm), containing six pots. Two pots, containing the oldest plants, were removed at weekly intervals and replaced with younger ones. The frequent replacement of pots of seedlings was done to avoid overcrowding. Care was also taken to keep the stock cultures of *M. dirhodum* free from other aphid species, parasitoids and predators. To obtain nymphs of uniform age, adult aphids were taken from the culture, transferred to the plants of the same treatment in a muslin cage and allowed to reproduce, usually for 24 h. The newly born aphid nymphs were then transferred to the experimental plants.

During these studies different biological parameters of aphids were studied i.e., development times, fecundity and aphid size.

Table 1. Stock and final nutrient solutions used for feeding potted wheat cultivars solutions based on Hewitt, 1952)

Compound	Stock solution ($g l^{-1}$)	Final solution (ml Stock solution l^{-1})	
$(NH_4)_2S0_4$	264.0	High	15.00
		Low	0.00
KCl	149.0	High	10.00
		Low	0.00
$NaH_2P0_4.2H_2O$	208.0	High	12.50
		Low	5.00
$MgS0_4.7H_20$	184.0		22.50
$CaCI_2.6H_2O$	182.6	High	44.00
		Low	5.00
Fe(EDTA)	36.4		2.22
Micronutrients			
$MnS0_4.4H_2O$	11.20		2.22
$CuS0_4.5H_20$	1.20		2.22
$ZnS0_4.7H_20$	1.50		2.22
H_3B0_3	0.18		2.22

Development Time

This was recorded by transfer of adult aphids into clip cages attached to the leaves of tested cultivars. After 24 h all the adults except one nymph were removed, thus leaving a single nymph in the clip cage. Clip cages were supported by sticks throughout the experiment. The remaining nymphs (one per cage) on all the seedling were allowed to grow on the plants until they had matured and begun to reproduce.

Fecundity

Aphid fecundity was assessed by counting the numbers of offspring born to an aphid caged on the leaf. The newly born nymph was released into a clip cage. When reproduction commenced, the clipped cages were inspected daily. Nymphs produced in the preceding 24h were counted and removed. This practice was continued for the ten days of reproduction (Wyatt and White, 1977). The position of the cages was changed every three to four days to minimize possible effects of plant damage on fecundity (Dewar, 1977). Each clip cage with one aphid individual was considered as one replicate.

Determination of the intrinsic rate of increase (r_m) values from data were calculated using a computer programme "Statspak" (van Emden 1993) based on Birch's (1948) equation and using an iterative calculation procedure. The input values consisted of: number of aphids started

as nymphs; numbers of nymphs surviving to reproduce; number of days over which reproduction was measured (in this case 10 days); mean numbers of days from birth to reproduction and daily fecundity.

Aphid Size

To determine aphid size, nymphs were reared in clip cages. After the fecundity had been assessed over 10 days, these aphids were removed from the plants and preserved in 70 % ethyl alcohol. Aphids were mounted on microscope slides and length of one hind tibia length of each mounted aphid was measured using a microscope eyepiece graticule (Hohmann et aI., 1988)

The growth indicators of parasitoids (development time, male and female pupal period, male and female hind tibial length and fecundity) reared on *M. dirhodum* parasitised at five days old aphids were also recorded. For this purpose newly emerged aphid nymphs were collected from each treatment and released on 20 days old plants of the same treatments (350-400 nymphs per treatment). These treatments (Plants having High Calcium: Low phosphorus and High Phosphorus: Low calcium) were placed separately in muslin covered cages. After five days of feeding on the plants, 20 aphids were randomly selected from each treatment and were preserved in 70% ethyl alcohol. The length of one hind tibia of each aphid was measured as a check on the effect of the treatments.

Six pairs of 24 h old mated parasitoids were introduced in each treatment cage for 48 h. When parasitized aphids became mummified, the mummies were removed and kept separately in glass vials. The total development time of 20 parasitoids from egg to mature larvae (i.e., from oviposition to mummification of the aphid) from each treatment was recorded. Male and female pupal period (days) were also recorded. Adults emerged from the mummies from each treatment were preserved separately in 70% ethyl alcohol. The one hind tibia length of 20 male and 20 female adult parasitoids were measured. The fecundity of randomly collected 15 female adult parasitoids fed 24 h on 30% honey-water solution immediately after killed in 70% ethyl alcohol was recorded in terms of the number of mature eggs present in the ovaries.

The data were analysed by analysis of variance as Completely Randomised with 20 replicates using a computer programme "Statspak"(van Emden, 1993) and the means were separated using Least Significant Differences (Steel and Torrie, 1960)

RESULTS AND DISCUSSION

The analysis of variance of growth indicators of aphids (development time, fecundity, intrinsic rate of natural increase and adult hind tibia length), revealed that there were no significant effects due to high calcium : low phosphorus or high phosphorus: low calcium (Table 2).

However, the hind tibia length of five days old aphid was significantly affected by phosphorus and calcium treatments. A greater hind tibia length (626.08 µm) was observed on plants fertilised with high phosphorus than with high calcium ($P < 0.05$). As with the aphids, the analysis of variance of most of the growth indicators of the parasitoid (development time, pupal period and

Table 2. Growth parameters of adult *M. dirhodum* reared on 'Rapier' fertilized with high calcium and high phosphorus

Growth indicators	High calcium	High phosphorus	F-ratio (1,27 df)
Development time (days)	10.61 a	10.56 a	0.025
Fecundity (number of nymphs produced in 10 days)	11.69 a	14.12 a	2.73
r_m	0.18 a	0.19 a	1.97
Hind tibia length (µm)	1259.20 a	1270.10 a	0.049

Means followed by the same letters do not differ significantly from one another at P < 0.05

Table 3. Hind tibia length of adult *M dirhodum* and growth parameters of A. rhopalosiphi reared on M. dirhodum parasitized at five day old aphids on 'Rapier' fertilized with high calcium and high phosphorus

Growth Indicators	High calcium	High phosphorus	F-ratio
Aphid			
Hind tibia length(μm)	575.12 a	626.08 b	8.95
Parasitoid			
Development time (days)	7.90 a	7.75 a	0.73
Male pupal period (days)	4.50 a	4.35 a	0.63
Female pupal period (days)	5.80 a	5.75 a	0.073
Male hind tibia length (μ m)	555.36 a	561.08 a	0.515
Female hind tibia length (μ m)	623.48 a	637.00 a	3.26
Fecundity	128.93 a	159.46 b	11.90

Means followed by the same letters do not differ significantly at P<0.05

male and female hind tibia length) also revealed no significant (P > 0.05) difference between the treatments. However, the fecundity of the parasitoid was higher (159.46 eggs per female) on plants fertilised with high phosphorus than on plants fertilised with high calcium (128.93 eggs) (P < 0.05), consistent with the larger aphids on that treatment (Table 3).

Ca and P were supplied in the form of $NaH_2PO_4.2H_2O$ and $CaCl_2.6H_2O.NaH_2PO_4.2H_2O$ was used as a source of P rather than commercial phosphate fertilisers e.g. single superphosphate or diammonium phosphate. Laboratory grade chemical $CaCl_2$ $6H_2O$ was used as a source of elemental Ca rather using the gypsum ($CaSO_4.5H_2O$), agricultural lime ($CaCO_3$) or $Ca(NO_3)_2$

It is evident from the results that high levels of calcium or phosphorus in the partially resistant host plant did not have a significant effect on the growth and development of aphids. However, five-day-old aphids were significantly bigger when reared on plants fertilised with high phosphorus as compared to those reared on plants fertilised with high calcium. These finding collaborate those of Barker and Tauber (1951) and Broadbent (1954), who reported the beneficial effect of high phosphorus fertilisation on aphids. Contrary to this EI- Tigani (1962) showed increases in the infestation of green peach aphid *(M. persicae)* and other aphids on plants given low or no phosphorus. Bonner (1951) found that calcium deficient plants were vulnerable to aphid *(M. persicae)* attack. The growth and development indicators of the parasitoid also indicated no significant effect of phosphorus or calcium on the development time, pupal period and size of the adult. However, the mean fecundity of the parasitoid was higher in plants fertilised with high phosphorus than with high calcium This is due to the fact that the bigger sized aphids produced bigger and more fecund parasitoids.

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