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Uptake and distribution of ^{14}C-labeled Fosthiazate in tomato (*Lycopersicon esculentum* L.)

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ABSTRACT

The uptake of ^{14}C-labeled fosthiazate (0.75 mg·L^{-1}) by tomato plants was studied in solution culture both in the presence or absence of 2,4-dinitrophenol (DNP, 1 × 10^{-2} mM), a metabolic inhibitor. Fosthiazate was rapidly taken up by tomato plants and nearly one third of the finally absorbed quantity was taken up in the first half an hour. The translocation of fosthiazate to the shoot part was under metabolic control during the initial stage of uptake. The kinetics of uptake both in the presence and absence of DNP conformed well to the dual phase than a single phase. In the presence of DNP, the uptake capacity (V_{max1}) for the initial phase suffered, approximately three fold reduction occurred in comparison to the absence of DNP while V_{max2} for the latter phase was statistically similar to the value observed in the absence of DNP signifying the metabolic dependence of the initial uptake phase. Autoradiography indicated that fosthiazate in the tomato plants tends to accumulate in the roots and at the root-shoot junction. In shoot, it is accumulated in the older leaves especially, near the leaf tip and margins.

Keywords: ^{14}C-Fosthiazate; Uptake; Translocation; Systemicity; Tomato

1. INTRODUCTION

Fosthiazate [(RS)-S-Sec-bentyle-O-ethyl 2 oxo 1,3-thiazolidin-3-yl phosphonothioate)] is a relatively new group non-fumigant, organophosphorus nematicide [1]. Studies in field plots have shown that fosthiazate exhibits similar efficacy as that other non-fumigant nematicides against a wide range of plant parasite nematodes, such as root knot nematode (*Meloidogyne* spp.), cyst nematodes (*Globodera* spp.) and root lesion nematodes (*Pratylenchus* spp.) [2]. It also has systemic activity against various species of insects and mites on the foliar part. Fosthiazate has been on the market in Japan since 1993 and is currently registered for use on potatoes for controlling cyst nematodes in the U.K. [3]. However, no published data are currently available on the uptake and translocation of ^{14}C-fosthiazate in tomato. Therefore, the present study was undertaken to study the uptake and translocation of (^{14}C) fosthiazate by intact tomato plants and to examine the metabolic dependence of these processes.

2. MATERIALS AND METHODS

2.1. Chemicals

The fosthiazate chemical was procured from Shanghai Institute of Chemical Industry Testing Centre, Shanghai, China. Radiolabelled ^{14}C-fosthiazate compound was procured from BRIT, Mumbai. All necessary chemicals used in the investigation were procured from E. Merck, Spectro Chem and Loba Chemicals, India

2.2. ^{14}C Uptake & Distribution through Solution Culture

Healthy seeds of tomato (var. Pant T-3) were sown in three plastic trays (45 × 30 × 7.5 cm) filled with washed quartz sand. After germination, 1/2 (half-strength) Hoagland solution was applied on alternate day for 2 weeks and later the plants were thinned to maintain 30 plants per tray. Thereafter, Hoagland solution of the full strength was applied thrice in a week. When the plants were 40 d old, plastic trays were filled with distilled water and plants were gently removed from the trays to ensure the minimum damage to the roots.

Exactly 150 ml Hoagland solution with or without DNP (1 × 10^{-2} mM) were taken in conical flasks of 250

ml capacity. To each flask 0.5967 μci of ^{14}C-fosthizate was added except for the control (with no fosthiazate). The final concentration of fosthiazate in the uptake solution was 0.75 mg/L. Two-40 d old-tomato plants were placed in each flask ensuring that their roots were properly dipped in Hoagland solution. The flasks were immediately tightly wrapped in black carbon paper to keep roots under dark. Plants were kept under the fluorescent light with the provision for aerating the solution. Plants were removed from duplicate flasks at 0.5, 1.0, 2, 4, 6, 8, 12 and 24 h periods. After removal, plants were washed in ice-cold tap water, Hoagland solution and distilled water, respectively and soak-dried between blotting paper sheets. One plant was kept for autoradiography and another was separated in roots and shoot parts and weighed on an electronic balance.

2.3. Solvent Extractant for Fosthiazate

The weighed root and shoot samples were weighed and ground separately in the presence of 10 ml of methanol: water (1:1) using a pestle and mortar. After grinding, the sample was transferred to a conical flask (100 ml capacity) using 20 ml methanol and extracted for 1 h on a mechanical shaker. The contents were filtered using Whatman No. 1 filter paper and 15 ml extract was passed through a column containing silica gel (2 g), and 5 g Na_2SO_4 and leached. The leachate was stored in glass vials.

2.4. Counting Procedure

One ml of leachate was taken in quartz glass scintillation vial and 9 ml of dioxane based scintillation cocktail (10 g PPO, 0.25 g POPOP, 100 g naphthalene/L of dioxane) was added. Prior to counting each sample was kept under dark for 10 h for the dark adaptation and to prevent false counts [4]. Each sample was assayed for the ^{14}C- labeled fosthiazate activity on a liquid scintillation counter (Pacard-1600 TR) using the external standardization method.

2.5. Autoradiographic Techniques

Tomato plants after uptake study were kept in herbarium sheets for drying. These plants were kept under the X-ray film in the cassettes for 15 d exposure time. Then negative films were subjected to positive method for clear visualization of accumulation and translocation of ^{14}C-labeled fosthiazate [5].

3. RESULTS AND DISCUSSION

The data on total amount of fosthiazate absorbed by the whole plants of tomato at different time intervals presented in **Figure 1** indicated that fosthiazate was rapidly taken up by tomato plants and under normal conditions nearly one third of the finally absorbed quantity was taken up in the first half an hour. The accumulation of fosthiazate in the tomato plants continued till the last observation (24 h).

The presence of 2,4-dinitrophenol (DNP), a metabolic inhibitor decreased the uptake of fosthiazate by tomato plants at all time intervals. The values of percent inhibition in the presence of DNP indicated that the relative inhibition was initially higher up to 2 h (44.68% to 59.66%) as compared to the later timings.Numerous investigations showed that carpropamid, a fungicide was rapidly absorbed and translocated by 14 d old rice seedlings in half an hour uptake period [6].

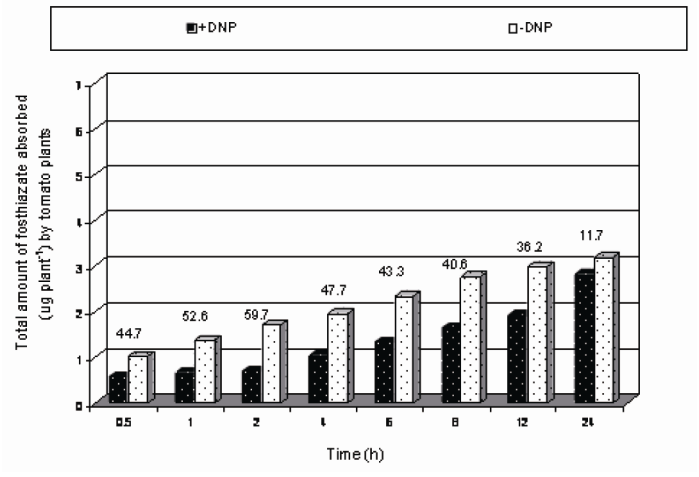

Figure 1. Total uptake of fosthiazate by tomato plants (ug·plant^{-1}) without or with 2,4-dintrophenol. The numerical values above the histograms indicate percent inhibition in the presence of DNP.

The data on percent distribution of absorbed fosthiazate in roots and shoot of tomato plants both with or without DNP are depicted in **Figure 2**. Under normal conditions (without DNP), the absorbed fosthiazate was easily translocated to the shoot part. However, in the presence of DNP most of the absorbed fosthiazate was retained in the roots during the initial stages of uptake and its translocation to shoot part was slowed down.

The distributions of radiolabelled fosthiazate in tomato roots (at 0.5 h) and in an intact tomato shoot (at 4 h) are depicted in an autoradiograph (**Plate 1**). As shown in the autoradiographs, the absorbed fosthiazate mainly accumulated in the roots and also at the root-shoot junction. The retention of fosthiazate in the stem was much lesser as compared to the leaves. In the shoot, the lower older leaves accumulated more fosthiazate as compared to the younger upper leaves. Within the leaf, fosthiazate concentrated at the leaf tip and also at leaf margins. The translocation of fosthiazate appeared to be mediated by the apoplastic movement which is solely regulated by physical forces like the transpirational pull and root pressure and leads to the accumulation of the compound at the apex and margins of leaves [7-9]. However, the observed partial inhibition of fosthiazate accumulation in the shoot in the presence of a metabolic inhibitor like DNP indicated that the translocation of absorbed fosthiazate to the shoot was partly dependent on the metabolism.

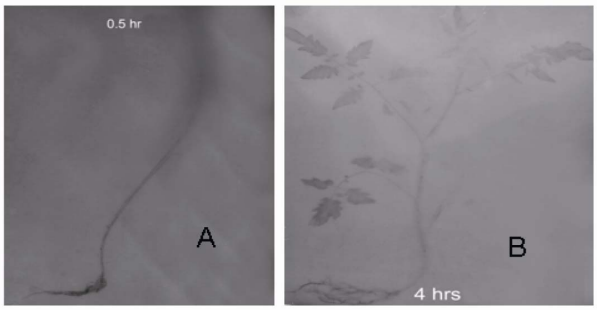

Plate 1. Radioautographs of ^{14}C-fosthiazate exposed tomato roots (A, after 0.5 h) and whole plant (B, after 24 h).

Time dependent kinetics of fosthiazate uptake by tomato plants both in the presence and absence of DNP was examined by using a modified Langmuir type equation (Equation 1).

$$\frac{1}{q} = \frac{1}{V_{max}} + \frac{B}{V_{max}} \cdot \frac{1}{t} \quad (1)$$

where,

q = amount of ^{14}C fosthiazate taken up by plant at time 't';

V_{max} = maximum absorption of ^{14}C fosthiazate/ plant;

B = a constant;

T = time (h).

The uptake data of fosthiazate by tomato plants were fitted to the Equation 1 by plotting the reciprocals of

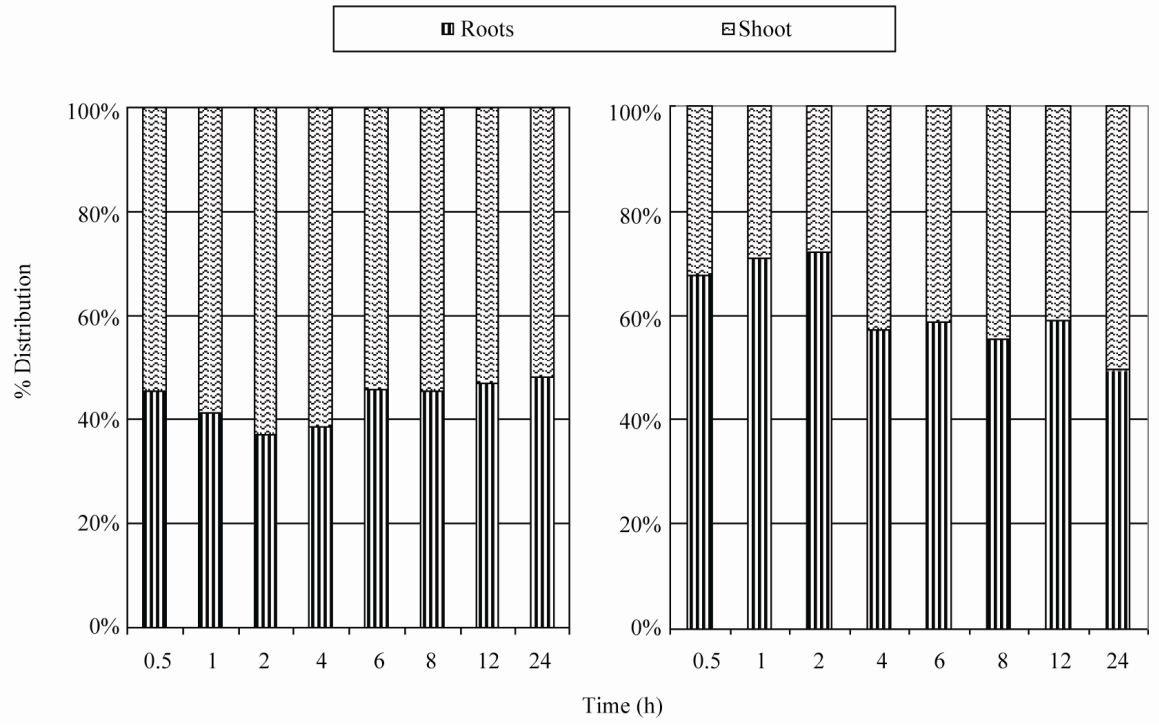

Figure 2. Percent distribution of fosthiazate between roots and shoot under both without and with DNP.

amount of fosthiazate (µg·plant^{-1}) absorbed by plants at a given time against time (h) **(Figure 3)**. Assuming that the kinetics of fosthiazate was governed by a single phase, the values of V_{max}, B and the coefficients of determination (R^2) for single phase were computed. However, a close perusal of the distribution of the points in **Figure 3** revealed that the uptake kinetics could be splitted into two distinct straight line parts; the first phase up to 2 h and the second one which appeared to operate beyond 2 h onward upto 24 h. The data of both the phases were fitted individually to the Equation 1.

The computed values of V_{max}, B and R^2 for both the single and dual phases for tomato plant are presented in **Table 1**. Statistically significant values of R^2-value indicated that the uptake data conformed to the kinetic equation used in the study. A comparison to R^2-values for the single and dual uptake phases revealed that the dual phase could account the uptake pattern of fosthiazate by tomato plants better than single phase both in the presence and the absence of DNP. In the presence of DNP, the value of V_{max} for the initial phase suffered a significant drastic (roughly three fold) reduction in comparison to the absence of DNP. On the other hand, the value of V_{max} for the latter phase in the presence of DNP was statistically similar to the value observed in the absence of DNP. This clearly indicated that the initial uptake of fosthiazate by tomato plants was under metabolic control.

In conclusion, this study showed that uptake of fosthiazate, a nematicide, by tomato plants was quite rapid suiting to its application through irrigation water. The kinetics of uptake both in the presence or absence of DNP conformed well to the dual phase than a single phase. The initial phase of uptake was under metabolic control. The initial translocation of fosthiazate to the shoot part was inhibited by metabolic inhibitor. In the tomato plants, fosthiazate tends to accumulate more in the roots and at the root-shoot junction. In shoot, it is accumulated in the older leaves especially, near the leaf tip and margins.

Table 1. Kinetic parameters of single and dual phase uptake of fosthiazate by roots and shoots of tomato plants with and without 2,4-dinitrophenol (DNP).

Model	Parameters	Without DNP	With DNP
Single phase	V_{max}	2.786	1.544
	B	0.931	1.050
	R^2	0.949**	0.751*
Dual phase (I)	V_{max1}	2.214	0.749
	B_1	0.598	1.680
	R_1^2	0.999**	0.991*
Dual phase (II)	V_{max2}	3.731	3.3934
	B_2	3.323	11.565
	R_2^2	0.926	0.994

**significant at p = 0.01 and *significant at p = 0.05.

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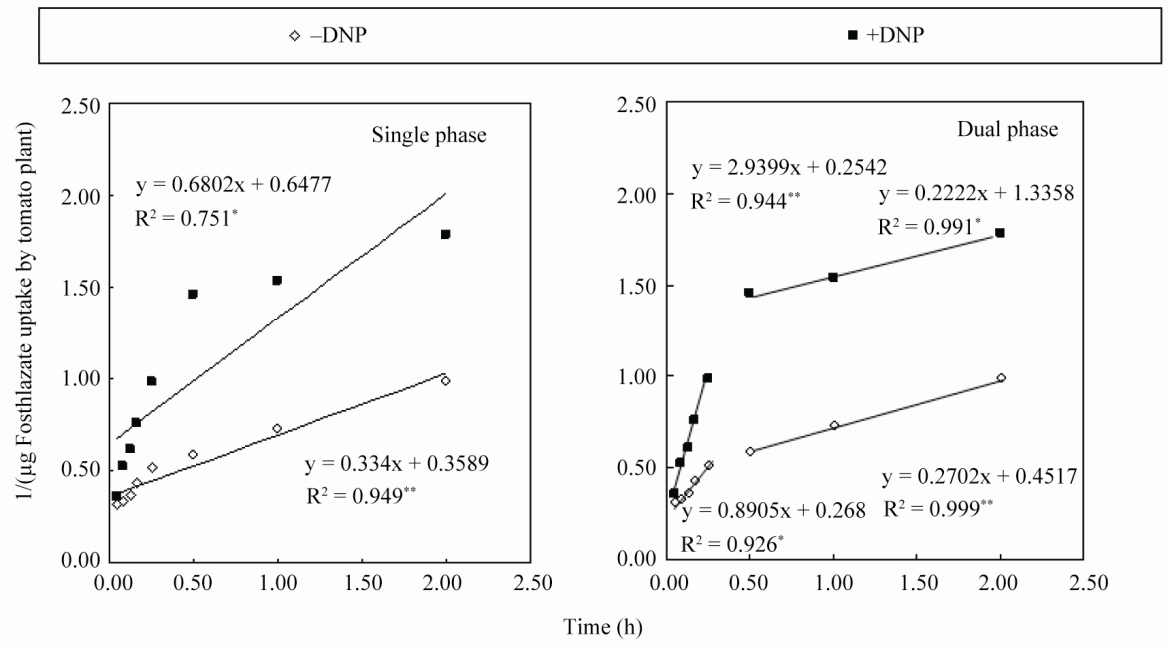

Figure 3. A plot of 1/q versus 1/t single and dual phase uptake of Fosthiazate by whole tomato plant. **Significant at p = 0.01, *Significant at p = 0.05.

necessary facilities.

REFERENCES

[1] Koyanagi, T., Imai, O. and Yoshida, K. (1998) Development of a new nematicide, fosthiazate. *Journal of Pesticide Science*, **23**, 174-177.

[2] Ingham, R.E., Hamm, P.B., Williams, R.E. and Swanson, W.H. (2000) Control of *Meloidogyne chitwoodi* in potato with fumigant and nonfumigant nematicides. *Journal of Nematology*, **32**, 556-558.

[3] Sturz, A.V. and Kimpinski, J. (1999) Effects of fosthiazate and aldicarb on populations of plant growth promoting bacteria, root lesion nematodes and bacteria-feeding nematodes in the root zone of potatoes. *Plant Pathology*, **48**, 26-32. doi:10.1046/j.1365-3059.1999.00305.x

[4] Horrock, D.L. (1974) Applications of liquid scintillation counting. Academic Press, New York, 331.

[5] John, D.H.O. and Field, G.T.J. (1963) A text book of photographic chemistry. Chapman and Hall, London, 29.

[6] Rohilla, R., Singh, U.S. and Singh, R.L. (2001) Mode of action of acibenzolar-S-Methyl against sheath blight of rice, caused by *Rhizoctonia solani* Kuhn. *Pest Management Science*, **58**, 63-66. doi:10.1002/ps.423

[7] Crowdy, S.H. (1977) Translocation in systemic fungicides in Book Systemic. In: Marsh, R., Ed., *Fungicides*, 2nd Edition, Longmann Ltd, New York, 92-114.

[8] Hammett, K.R.W. (1968) Root application of systemic fungicides for control of powdery mildews. *Plant Disease Report*, **52**, 754-760.

[9] Singh, U.S., Kumar, J. and Tripathi, R.K. (1985) Uptake, translocation, distribution and persistence of 14C-metaxyl in pea (*Pisum sativum*) Z. *Pflanzenkrankh Pflanzenschutz*, **92**, 64-67.

