Translocation and distribution of 32P labelled potassium phosphonate in black pepper (Piper nigrum L)

Article *in* Crop Protection · October 2009

DOI: 10.1016/j.cropro.2009.06.016

6 authors, including:

Velayudhan kt
Centre for Water Resources Development a…

Suseela Bhai
Indian Institute of Spices Research

Translocation and distribution of ^{32}P labelled potassium phosphonate in black pepper (*Piper nigrum* L)

R. Anil Kumar [a,*], K. Vasu [b], K.T. Velayudhan [b], V. Ramachandran [c], R. Suseela Bhai [d], G. Unnikrishnan [a]

[a] *Polymer Science and Technology Laboratory, Department of Science and Humanities, National Institute of Technology- Calicut, Kozhikode, Kerala - 673 601, India*
[b] *Isotope Hydrology Division, Centre for Water Resources Development and Management, Kozhikode, Kerala - 673 571, India*
[c] *Nuclear Agriculture and Biotechnology, Division Bhabha Atomic Research Centre, Mumbai - 400 085, India*
[d] *Division of Crop Protection, Indian Institute of Spices Research, Kozhikode, Kerala - 673 012, India*

A B S T R A C T

The aim of this project was to study the translocation of potassium phosphonate within black pepper (*Piper nigrum* L.) and to elucidate the amount of chemical distributed through out the plant based on a tracer technique. Potassium phosphonate is a potential fungicide used against the pathogen *Phytophthora capsici*, which causes *Phytophthora* foot rot (Quick wilt) in the plant. Reports indicate that translocation of phosphonate is ambimobile, but there is no visual evidence for this. Potassium phosphonate labelled with radioactive phosphorus, ^{32}P, was applied to the black pepper vine and bush pepper and its translocation to different parts of the pepper plant was studied using an autoradiography technique. The chemical which migrated to different parts of the plant was quantitatively estimated by measuring ^{32}P on a liquid scintillation spectrometer following Cerenkov counting. Only traces of the chemical were lost to the soil through root systems. These results indicate that foliar sprays of potassium phosphonate to pepper could be a viable method of application for soil and foliar pathogens.

Keywords:
Autoradiography
Black pepper
Liquid scintillation counting
Phosphorus-32
Potassium phosphonate
Phytophthora capsici

1. Introduction

Black pepper (*Piper nigrum* L.) is a climber and the hot, humid climate of submountainous tracts of Western Ghats, India is ideal for its growth. Among all diseases that affect black pepper, *Phytophthora* foot rot (Quick wilt) caused by *Phytophthora capsici*, a soil borne water Oomycete, is the most destructive (Sarma et al., 1991). *P. capsici* has a wide host range including horticultural and spice crops (Erwin and Ribeiro, 1996). The disease occurs mainly during the south-west monsoon season (June–September). All parts of the vine are vulnerable to infection. Losses were estimated at 5% to 20% annually in Malaysia, India, Indonesia and Vietnam (Drenth and Sendall, 2004, pp. 10–28).

Different types of fungicides are used the world over for the control of root diseases caused by species of *Phytophthora*. Soil drenching, trunk injection and foliar spraying are different modes of application of these fungicides. For horticultural crops, other than tree crops, trunk injection is not possible and hence either soil drenching or foliar spraying or both are the preferred mode of application (Allen et al., 1980; Darvas et al., 1984; Pegg et al., 1985; Magarey et al., 1991). Most of the systemic fungicides are limited to either acropetal (upward) or basipetal (downward) movement within the plant thus limiting their applications. For example, xylem-translocated fungicides (e.g., benomyl, carbendazim, metalaxyl etc.) move acropetally in the transpiration stream to the leaves (Edgington and Peterson, 1977, pp. 51–89; Staub et al., 1978). However, if translocation is limited to apoplastic movement regulated by physical factors such as root pressure and transpiration, fungicides tend to accumulate only in leaf apices and margins (Singh et al., 1985). Metalaxyl has acropetal systemic mobility and although it is effective against root infections when applied as a soil drench, it has no activity against the same when applied as a foliar spray (Staub et al., 1978).

If the fungicide is translocated in the symplast as well as the apoplast, it will be xylem and phloem translocated, and will move both acropetally and basipetally in the plant (Bertrand et al., 1977; Ouimette and Coffey, 1990; d'Arcy-Lameta and Bompeix, 1991). The phosphonate range of fungicides fulfills the above criteria enabling a wider range of plant diseases to be controlled by a single product. Ouimette and Coffey (1990) demonstrated that phosphonate had near-identical translocation profiles to [^{14}C] sucrose, suggesting an

* Corresponding author. Tel.: +914952286560, +919986386014; fax: +914952286103.

E-mail addresses: anilmuktharam@yahoo.co.in (R. Anil Kumar), vasuk_09@rediffmail.com (K. Vasu), kt_velayudhan@rediffmail.com (K.T. Velayudhan), vrchand@magnum.barc.ernet.in (V. Ramachandran), rsbhai@rediffmail.com (R. Suseela Bhai), unnig@nitc.ac.in (G. Unnikrishnan).

active mechanism for symplastic entry of this molecule. This is not surprising as the anion is chemically similar to phosphate, which is actively transported across membranes (Wojtaszek et al., 1966). Potassium phosphonate, a mixture of mono- and di-potassium salt of pentavalent phosphonic ($H_3P(O)O_2$) acid is one such product.

Preliminary investigations have shown that an aqueous solution of potassium phosphonate is effective against *Phytophthora* foot rot in black pepper (Veena and Sarma, 2000). The fungicide is applied both as a foliar spray and soil drench. Only scanty information is available on the translocation and redistribution of the applied fungicide in the plant. Hence the objective of the present investigation was to study the translocation and distribution of potassium phosphonate in black pepper using radioactive ^{32}P. The results are presented in the following sections.

2. Materials and methods

2.1. Establishment of experimental plants

Black pepper (*P. nigrum* L.) vines for the experiment were raised in the green house at the Centre for Water Resources Development and Management (CWRDM), Kozhikode, Kerala, India. *Erythrina indica* was planted adjacent to each pot to serve as standard to the pepper. Black pepper vines (var. *Panniyur-1*) were planted in earthen pots (30 cm diameter) filled with potting mixture (1:1:1 soil: sand: farmyard manure) and trailed on to the standard. The plants were irrigated daily using a drip irrigation system. Similarly, black pepper laterals popularly known as bush pepper (var. *Karimunda*) were also raised in earthen pots. Similar agricultural practices as for the pots above, like irrigation and manuring were followed.

2.2. ^{32}P- labelled potassium phosphonate and its application to the pepper plants

Potassium phosphonate labelled with radiophosphorus containing 10 mCi (370 MBq) of the tracer with a specific activity of 2 mCi ml^{-1} was procured from the Board of Radiation and Isotope Technology (BRIT), Mumbai, India for field application. The synthetic route for the preparation of ^{32}P- labelled potassium phosphonate adopted by BRIT (Asokan et al., 2004) is as follows:

$$H_3{}^{32}PO_4 + 3PCl_5 \xrightarrow{-65^\circ C} 4\,{}^{32}POCl_3 + 3HCl$$

$$^{32}POCl_3 \xrightarrow[650^\circ C]{C} {}^{32}PCl_3 \xrightarrow{H_2O/KOH} KH_2{}^{32}PO_3$$

Two ml (equivalent to 4 mCi ^{32}P) of the labelled potassium phosphonate was diluted to 1 l with potassium phosphonate solution used as a carrier. The final solution had a concentration of 1200 mg HPO_3^{2-} l^{-1} and contained 4 μCi ^{32}P ml^{-1}.

2.3. Foliar application of labelled potassium phosphonate to black pepper vines

Eighteen, one-year-old pepper vines, approximately 2 m high, were selected for foliar application. Diluted radioactive solution was smeared, using an artists' brush, on to the third or fifth leaf, from the top which is about 2 m above the collar of the vine. The chemical was applied to the lower surface of the leaf, for more effective absorption by the plant.

2.4. Preparation of the plant material for autoradiography

One treated vine was uprooted at an interval of a day for 18 d. The roots were washed in tap water and dried using tissue paper. The treated plant was pressed between blotting paper for 2–3 d. The plant was cut into four portions, viz., top, middle, lower and root portions. Each part was placed on blotting paper and fixed using small pieces of cellophane tape. The paper was placed over an X-ray film cassette (Ultra Kiran, India) and in a dark room under a red safety lamp, autoradiography film (X-ray film; Kodak) was placed on top of the pressed plant sample. The cassette was locked and wrapped in a thick black polythene cover and stored at −20 °C for 1–2 weeks.

2.5. Development and fixing of autoradiograph

The exposed film was removed from the cassette in a dark room under a red safety lamp. The film, suspended on a hanger and was placed in the developer (Premier, India) and swirled gently for about 2–3 min. The film was then removed from the developer and rinsed with tap water before being placed in the fixer (Premier, India) and swirled gently for 2–3 min. The film was finally washed

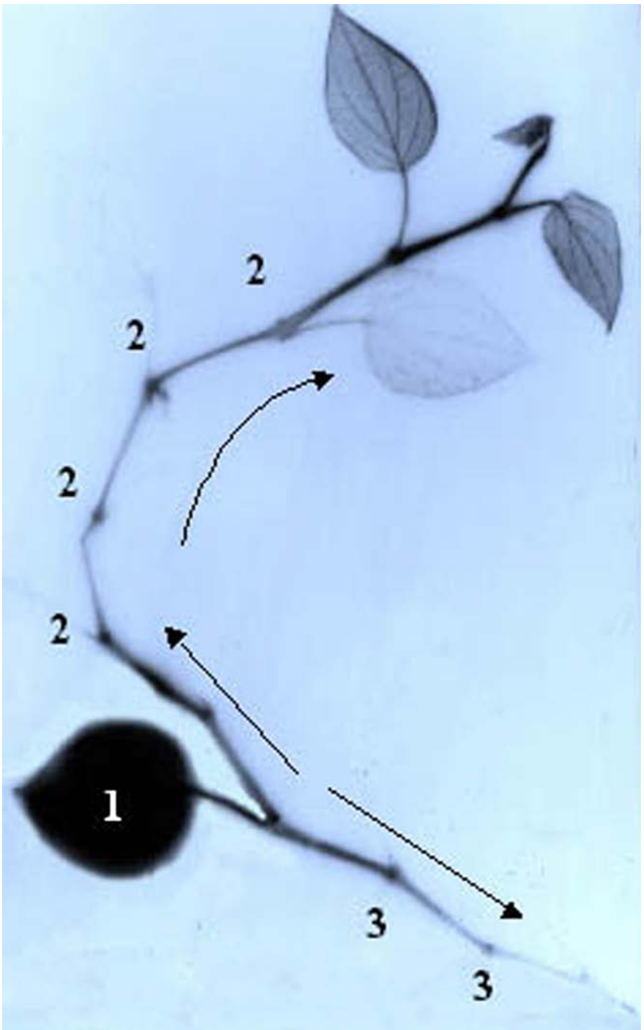

Fig. 1. Upward [2] and downward [3] movement of ^{32}P labelled potassium phosphonate from the point of application [1] in black pepper.

in running tap water for about 5 min and allowed to drip dry. The dried X-ray film was removed from the hanger and labelled.

2.6. Quantification of the tracer in different parts of the pepper plant and soil

Six, one-year-old bush pepper plants bearing pepper berries were used for this study. Diluted radioactive solution was smeared on to the lower surface of one of the leaves in three bush pepper plants. The other three plants were served as control. All the plants were uprooted on the 3rd d and separated into leaves, stem, berry and root. The leaf to which radiochemical was applied was thoroughly washed with distilled water to remove excess chemical and kept in a paper bag separately. All other parts were washed with distilled water and kept in labelled paper bags. The paper bags were kept in hot air oven at 60 °C for 2 d to dry the plant material. The latter were then ground to fine powder and stored. The soils around the uprooted plants were also collected. They were air dried in shade, passed through a 2 mm sieve and stored. All samples were stored at 2 –8 °C prior to their analysis (within 2 d).

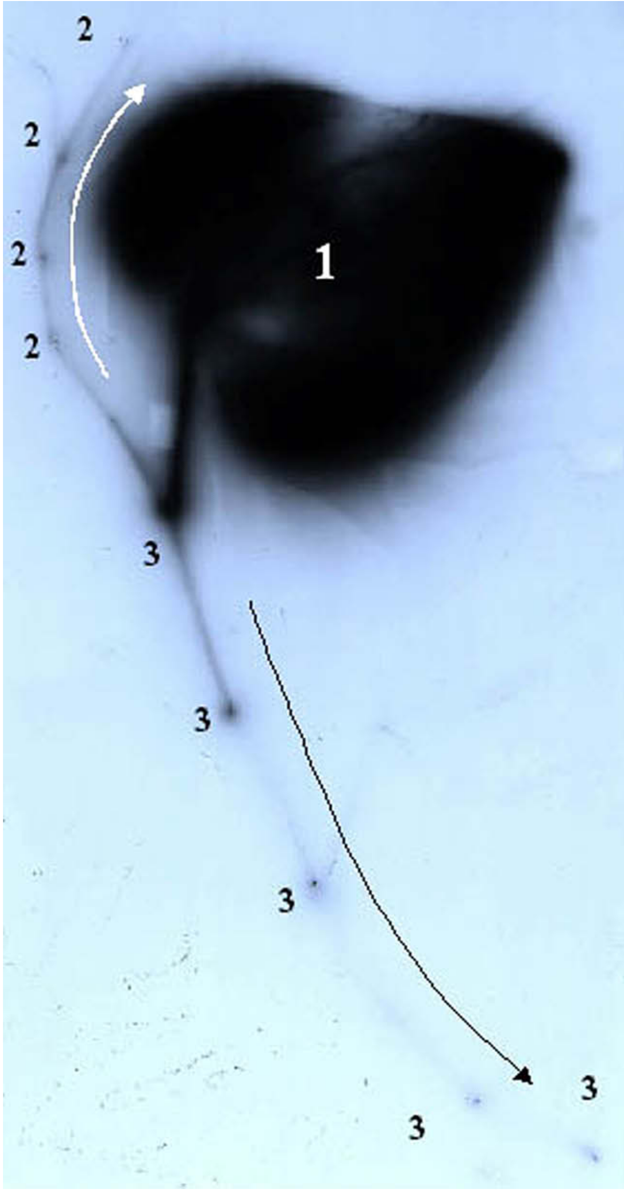

Fig. 2. Upward [2] and downward [3] movement of ^{32}P labelled potassium phosphonate from the point of application [1] in black pepper.

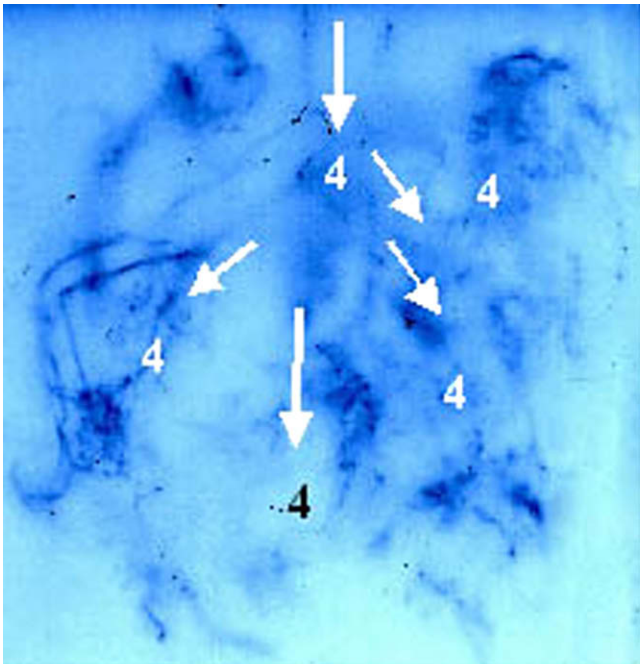

Fig. 3. Downward movement of the radiolabelled chemical to the root [4] of black pepper vine.

One gram of plant material was weighed into a 100 ml conical flask to which 15 ml of diacid (1:1 perchloric-nitric acid mixture) was added and digested till a colourless liquid was obtained. Digested samples were filtered, transferred to 25 ml standard flasks and made upto the mark with distilled water. Twenty milliliters of the extract were drawn into a scintillation vial and counted in a liquid scintillation counter (Wallac 1400, Finland) following Cerenkov counting. In the final calculations, the reading of the control was subtracted from the reading of samples and the activity of ^{32}P expressed as counts per minute (CPM) g^{-1} of sample.

Similarly, 5 g of air-dried soil was digested with 25 ml of diacid in a 100 ml conical flask fitted with a funnel, to a colourless solution. The extract was filtered, transferred to a 50 ml standard flask and made up to the mark with distilled water. Twenty milliliters of the extract were drawn into a scintillation vial and counted on a liquid scintillation counter following Cerenkov counting. The concentration of ^{32}P was expressed as CPM g^{-1} of soil after effecting the correction for background.

2.7. Statistical analysis

The radiolabelled potassium phosphonate was not only successfully mapped the translocation of the applied chemical to various parts of the pepper plant, but was also used to quantify the amount of phosphonate present at each site in terms of the specific

Table 1
Distribution of radiolabelled phosphonate in different parts of bush pepper plant and in surrounding soil.

Replicate No.	Specific activity of ^{32}P (CPM g^{-1} of sample ± SE)					
	Treated leaf	Leaf	Stem	Berry	Root	Soil
1	16822 ± 301	19 ± 3	278 ± 37	336 ± 4	39 ± 16	2 ± 1
2	41251 ± 552	9 ± 6	314 ± 16	358 ± 17	46 ± 4	2 ± 1
3	33243 ± 220	27 ± 14	413 ± 5	657 ± 24	67 ± 16	4 ± 1

Table 2
Percentage distribution of radiolabelled phosphonate in different parts of bush pepper plant and in surrounding soil

Replicate No.	Leaf		Stem		Berry		Root		Soil		Total
	*[A]	%	*[A]	%	*[A]	%	*[A]	%	*[A]	%	*[A]
1	19	2.82	278	41.25	336	49.85	39	5.79	2	0.30	674
2	9	1.23	314	43.07	358	49.11	46	6.31	2	0.27	729
3	27	2.31	413	35.36	657	56.25	67	5.74	4	0.34	1168
Mean		**2.12**		**39.89**		**51.74**		**5.95**		**0.30**	

*[A] - Specific activity of ^{32}P (CPM g^{-1} of sample).

activity of the radionuclide. The activity readings of ^{32}P represent means along with the standard errors, measured directly from the instrument. The results have been expressed as the percent radioactive phosphonate at a particular site by the method of Wahid et al., 1988:

$$\text{Percentage radioactive phosphonate at a particular site} = \frac{(\text{Specific activity of } ^{32}\text{P at a particular site}) \times 100}{\text{Total of the specific activity from each site}}$$

3. Results and discussion

Through the autoradiographic technique, it was found that phosphonate is rapidly translocated in the pepper plant consistent with apoplastic and symplastic transport (Figs. 1 and 2). The chemical moves further down to the roots within 5 d of its application (Fig. 3).

The distribution of ^{32}P labelled phosphonate, 3 d after application, in different parts of bush pepper plant is given in Table 1. Apart from the leaf where the tracer was applied, the chemical is concentrated relatively more in the stem [39.89%] and the berries [51.74%] (Table 2). It was also observed that by foliar application, only a negligible amount [0.30%] of the applied chemical reaches the soil (Table 2).

Usually the chemical is applied just at the onset of south-west monsoon in the pepper growing regions and coincides with the flowering and fruit bearing stages, which are prone to attack by the pathogen. The concentration of the chemical in the stem should obviously be high because of its ambimobile nature although it was more concentrated in the berries, the metabolic sink. Since the mammalian toxicity of phosphonate anion is similar to phosphate and less than aspirin (Dunhill, 1990), and there is about six months' interval between the chemical application and harvest, the high initial concentration of chemical in the fruit should not cause consumption problems for the end user.

A foliar spray of potassium phosphonate to pepper can be an effective method of disease control because the loss of the chemical is minimal during application. Moreover, foliar application is more economical than soil drenching as the chemical is not concentrated in a particular region as in soil drenching and its systemic movement ensures that vulnerable plant parts are protected.

Acknowledgements

The authors are thankful to the Executive Director, Centre for Water Resources Development and Management, Kozhikode, Kerala, India for the encouragement. They are also thankful to the Board of Research in Nuclear Sciences, Department of Atomic Energy, Government of India, Mumbai, India for the financial assistance.

References

Asokan, K.P., Sebastian, J., Jayachandran, N., Unny, V.K.P., 2004. Facile preparation of radiolabelled potassium phosphonate. J. Radioanal. Nucl. Chem. 262 (3), 767–769.
Allen, R.N., Pegg, K.G., Forsberg, L.I., Firth, D.J., 1980. Fungicidal control in pineapple and avocado diseases caused by *Phytophthora cinnamomi*. Austral. J. Exp. Agric. Anim. Husb. 20, 119–124.
Bertrand, A., Ducret, J., Debourge, J.C., Horriere, D., 1977. Etude des propriétés d'une novelle famille de fongicides: les monoéthylphosphites métalliques caractéristiques physico-chimiques et propriétés biologiques. Phytiatr. Phytopharm. 26, 3–18.
d'Arcy-Lameta, A., Bompeix, G., 1991. Systemic transport of tritiated phosphonate in tomato plantlets (*Lycopersicon esculentum* Mill.). Pestic. Sci. 32, 7–14.
Darvas, J.M., Toerien, J.C., Milne, D.C., 1984. Control of avocado root rot by trunk injection with phosetyl-Al. Plant Dis. 68, 691–693.
Drenth, A., Sendall, B., 2004. Economic impact of *Phytophthora* diseases in Southeast Asia. In: Drenth, A., Guest, D.I. (Eds.), Diversity and Management of *Phytophthora* in Southeast Asia, pp. 10–28. ACIAR Monograph, No. 114.
Dunhill, R.H., 1990. The manufacture and properties of phosphonic (phosphorous) acid. Austral. Plant Pathol. 19, 138–139.
Edgington, L.V., Peterson, C.A., 1977. Systemic fungicides: theory, uptake and translocation. In: Siegel, M.R., Sisler, H.D. (Eds.), Antifungal Compounds, vol. 2. Marcel Dekker, New York, pp. 51–89.
Erwin, D.C., Ribeiro, O.K., 1996. *Phytophthora* Diseases Worldwide. The American Phytopathological Society, APS Press, St. Paul, Minnesota.
Magarey, P.A., Wachtel, M.F., Newton, M.R., 1991. Evaluation of phosphonate, fosetyl-Al and several phenylamide fungicides for post-infection control of grapevine downy mildew caused by *Plasmopara viticola*. Austral. Plant Pathol. 20, 34–40.
Ouimette, D.G., Coffey, M.D., 1990. Symplastic entry and phloem translocation of phosphonate. Pestic. Biochem. Physiol. 38 (1), 18–25.
Pegg, K.G., Whiley, A.W., Saranah, J.B., Glass, R.J., 1985. Control of *Phytophthora* root rot of avocado with phosphorous acid. Austral. J. Plant Pathol. 14, 25–29.
Sarma, Y.R., Ramachandran, N., Anandaraj, M., 1991. Black pepper diseases in India. In: Sarma, Y.R., Premkumar, T. (Eds.), In: Diseases of Black Pepper. Proceedings of the Int. Pepper Comm. Wkshop. in Jt. Res. for Control of Black Pepper Diseases, 27–29 October 1988, Goa, India.
Singh, U.S., Kumar, J., Tripathi, R.K., 1985. Uptake, translocation, distribution and persistence of ^{14}C - metalaxyl in pea (*Pisum sativum*). Plant Dis. Prot. 92, 164–175.
Staub, T., Dahmen, H., Schwinn, F.J., 1978. Biological characterisation of uptake and translocation of fungicidal acylalanine in grape and tomato plants. Z. Pflanzenkr. Pflanzenschutz. 85, 162–168.
Veena, S.S., Sarma, Y.R., 2000. Uptake and persistence of potassium phosphonate and its protection against *Phytophthora capsici* in black pepper. In: Spices and Aromatic Plants: Challenges and Opportunities in the New Century. Contributory Papers, Centennial Conference on Spices and Aromatic Plants, Calicut, Kerala, India, 20–23 September 2000, pp. 243–248.
Wahid, P.A., Kamalam, N.V., Sankar, S.J., 1988. A device for soil injection of ^{32}P solution in root activity studies of tree crops. J. Plant. Crops 16, 62–64.
Wojtaszek, T., Cherry, J.H., Warren, G.F., 1966. Effect of 4,6-dinitro-o-sec-butylphenol on phosphorus accumulation and incorporation into tomato leaf discs. Plant Physiol. 41, 34–38.