

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

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ABSTRACT

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.

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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

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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 ($\text{H}_3\text{P}(\text{O})\text{O}_2$) 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.

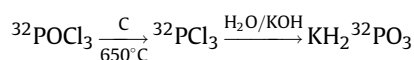
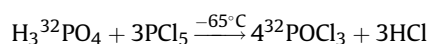
2. Materials and methods

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:



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 $\text{HPO}_4^{2-} \text{ l}^{-1}$ and contained 4 $\mu\text{Ci } ^{32}\text{P ml}^{-1}$.

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

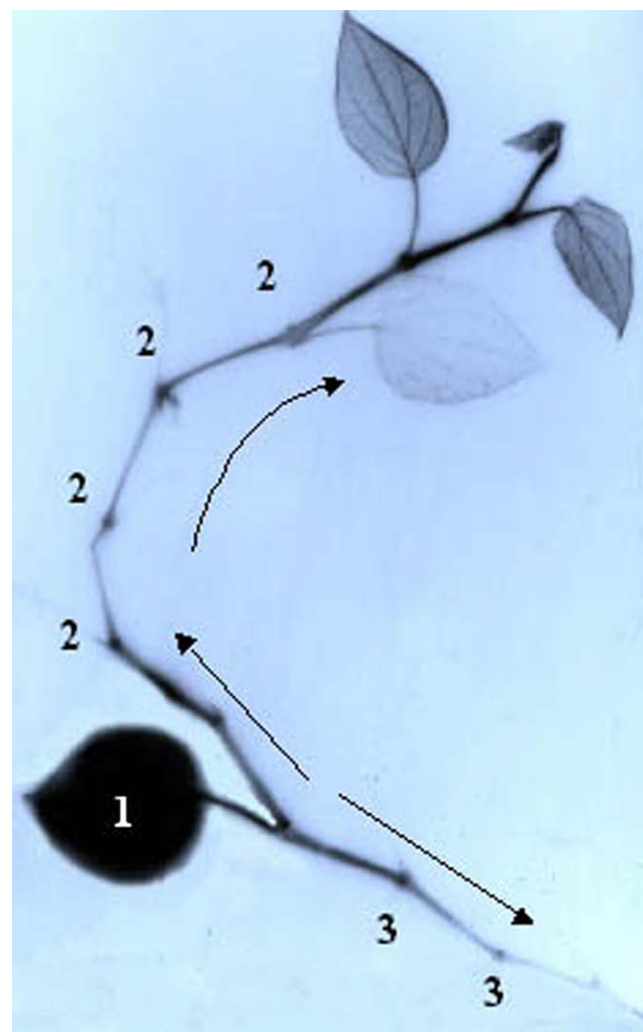


Fig. 1. Upward [2] and downward [3] movement of ^{32}P labelled potassium phosphonate from the point of application [1] in black pepper.

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