Residue Analysis of Triadimefon in Wheat by Using Test Fungus and Thin Layer Chromatography

KALPNA BHATNAGAR, B.B. LAL THAKORE, SNEH MATHUR AND B.P. CHAKRAVARTI

바트나가르 K.•B.B. 랄타코레•S. 마투르•B.P. 차크라바티 : 薄層 크로마토그라피와 指標 곰팡이의 利用에 의한 밀에 있어서 Triadimefon의 잔류량 분석

Korean J. Plant Prot. 25(4) : 229~233(1986)

ABSTRACT By using the test fungus *Macrophomina phaseolina*, residues of triadimefon were found in straw collected after harvest from sprayed plants of wheat varieties Kharchia and Lal Bahadur but grains contain no such residues. Thin layer chromatographic method was developed to detect residues of the fungicide which was found to be present in straw of sprayed plants of both the varieties. No residues could be detected in grain samples. It was found that triadimefon was converted in triadimenol in/to host.

INTRODUCTION

Persistence and residue problems with systemic fungicides are different from those of nonsystemic fungicides. Systemic fungicides are absorbed by the plant, translocated in different parts within the system and thus suffer less surface weathering. Triadimefon, a new systemic fungicide has been reported to control various diseases of wheat including rusts (Jain *et al.* 1980). There are several systemic fungicides which are effective in controlling different plant diseases but in absence of sufficient residue analysis data, these could not be recommended for commercial use in different crops. Hence, an attempt has been made for detection of residues by test fungus and thin layer chromatography of triadimefon; results of which are presented in this paper and briefly reported earlier (Baxi *et al.* 1982).

MATERIALS AND METHODS

Wheat varieties Kharchia and Lal Bahadur which are susceptible to yellow, brown and black rusts were used throughout the experiments. These wheat varieties were grown in field in the month of November 1980 and were sprayed with different concentrations of triadimefon i.e., 0.05, 0.1 and 0.2 per cent at the boot stage. For all experiments Triadimefon (Bayleton 25 WP, 1-(3 chlorophenoxy) -3, 3-dimethyl-1-1H-(1, 2, 4 triazol-1-yl)-2 butanone and triadimenol Baytan 15SD, i-(4 chlorophenoxy)-3, 3-dimethyl-(1,2,4 triazole-1-yl)-2 butanole) by M/S Bayer India Ltd. were used. Bioassay of triadimefon as well as triadimenol was done against test fungus *Macrophomina phaseolina* and standard curves were drawn to compare the residues in plant parts(Fig. 1 & 2). The concentration of fungicide in straw and grain was calculated

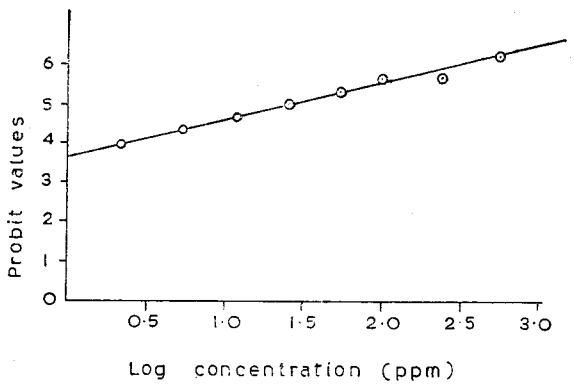

Fig. 1. Standard curve of growth inhibition of test fungus *Macrophomina phaseolina* against Bayleton.

Department of Plant Pathology, Rajasthan College of Agriculture, Sukhadia University, Udaipur (Raj.), India(인도 Sukhadia 대학, Rajasthan 농대 식물병리학과)

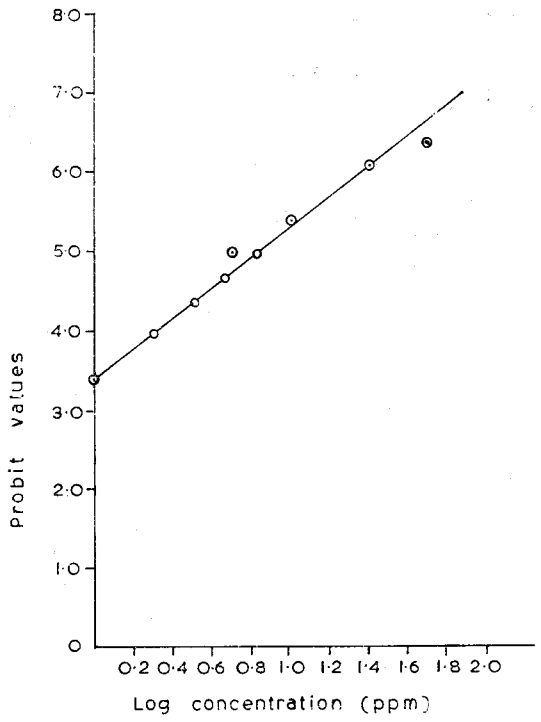

Fig. 2. Standard curve of growth inhibition of test fungus *M. phaseolina* against Baytan.

from standard curve of growth inhibition of test fungus against triadimefon and triadimenol. In standard curve percent growth inhibition was transformed in probit values(Finmey, 1981) and the concentration(ppm) was converted to logarithmic value. The inhibition of mycelial growth, expressed as probits, was plotted against log dose of the fungicides from the curve the residues were calculated.

Eight samples of straw and grain were collected from each treatment (i.e., 0.05, 0.1 & 0.2%) along with control of both the varieties at the time of harvesting. Fifty gram samples were crushed with 20 ml of water plus 180 ml of acetone and kept for one hour for commete extraction of fungicide from plant tissues. The material was filtered through Whatman filter paper No. 1 and then centrifuged, the supernatant collected in 150 ml flask, evaporated to dryness on hot water bath after adding 20ml Potato Dextrose Agar. These flasks were sterilized (Baxi *et al.* 1982). The media were poured in petri plates and inoculated with test fungus. Experiments were repeated thrice.

In case of thin layer chromatography the extraction was done similarly except that instead of PDA, 20ml of acetone was added to dissolve the residues. In case of straw, chlorophyll was removed by adding charcoal powder.

For preparing TLC plates, all glassware including glass plates(3″×6″) were first washed with detergent powder and then with glass distilled water. Slurry was prepared by suspending 40gm silica gel G in 100ml of chloroform and methanol mixture (60 : 40 v/v). Slurry was stirred well before use. Two plates which were joined back to back by adding a drop of acetone were dipped in the slurry and then were separated from each other with gel coated on one side of each plate. The coated slides were air dried for 5 minutes at room temperature and then dried at 50C.

Extract was pplied on the coated plates to each point with the help of micropipette. The plate was then placed in saturated speciman jar (30×10cm) containing solvent mixtures. The chromatogram was allowed to run a distance covering three fourth length of plate. The plate was removed and air-dried for 5 minutes. The slides were sprayed with colouring reagent with the help of an atomizer to develop the spot. To calculate Rf value measurement of distance travelled by solute and distance travelled by solvent was taken.

RESULTS

Residue estimation by using test fungus. Average results of three trials are given in Table 1. At 0.1% concentration of triadimefon spray, residues in straw of Lal Bahadur and Kharchia were respectively 0.11 ppm/gm and

0.05 ppm/gm of tissues and at 0.2% these values were 0.46 ppm/gm and 0.23 ppm/gm of tissues. No residues were found either in grain or in straw at 0.05% concentration of the fungicide. There was no residue of triadimefon in grains of the two wheats in any of these experiments. As apparent from standard curve of triadimenol (Baytan), when leaves were sprayed with 0.1% triadimefon, the residues were 0.04 ppm/g and 0.05 ppm/g of tissues in case of Kharchia and Lal Bahadur respectively while in case of samples taken from sprayed plants with 0.2% triadimefon, results were respectively 0.09 ppm/gm and 0.13 ppm/gm of tissues but no residuss were detected in grains and straw at 0.05%.

Residue analysis by thin layer chromatography. Among different solvent mixtures Benzene-Acetone-Water (90 : 10 : 10) was found best. These three compounds were mixed thoroghly in a flask up to its saturation and then mixture was kept for setting. Upper layer containing acetone-benzene mixture saturated with water was collected and used as solvent while the lower layer of water was discarded.

Triadimefon standard (10μmg) was applied on silica coated glass slides. These slides were kept in small jars containing solvent and were allowed to run up to 3/4 of coated slides. Then these slides were removed, air-dried and kept in jar saturated with iodine vapours. A dark brown colour spot was formed on slide in case of triadimefon, but in treatment the iodine vapours failed to develop the spot. In another experiment glass plates (6″×3″) were coated with silica gel, triadimefon and triadimenol standards (0.81%) were prepared and 10, 20, 30, 40, 50 and 100μml amount of these solvents were spotten on these plates. For spot development, 3 reagents viz., iodine vapours, KI-I2 solution and 2% KMnO4 were used. KI-I2 solution did not develop the spot at lower concentration i.e., 50μmg but 2% aqueus solution of KMnO4 when sprayed on slide while still moist, a white shining spot appeared on the slide after 10 minutes of spraying. Using this spray reagent triadimefon was detected up to 10μmg concentration whereas a dark brown spot developed when iodine vapour was used. The Rf value of triadimefon in both the colour developers was 0.7. But no spot developed in treatment when used in iodine vapours, whereas white spot developed when KMnO4 was used as colour developer. The Rf value was 0.35. Spot developed towards the lower side of the plate and Rf value was 0.35 while spot due to triadimefon developed towards upper side and the Rf value was 0.7. In case of treatments, the spot developed at the same distance as in case of triadimenol standard, and Rf value was 0.35.

From these findings it is inferred that after absorption in plant tissue triadimefon is converted to its metabolite which resembles triadimenol. No residue of triadimefon could be detected in any of the grain samples which were sprayed with 0.05, 0.1 and 0.2 per cent concentrations of fungicide. Spot did not appear in straw taken from plants sprayed with 0.05 and 0.1% concentrations but light spot developed at higher concentration of extract taken from these plants. Very shining white spot developed which persisted for longer period when extract from plants sprayed with 0.2% were taken indicating the presence of residues.

DISCUSSION

Some workers have studied the degradation of systemic fungicides and their residue present in plant products by the use of test fungi, thin layer chromatography, bioautography, colorimetry and gas chromatography methods.

In present investigations test fungi and thin layer chromatography methods were employed to find out the residue of triadimefon. These methods have been used by various workers for residue analysis of different systemic fungicides (Tripathi *et al.* 1976, Tafuri *et al.* 1978 and Waring & Wolfs 1975).

Besides using test fungus for residue studies of systemic fungicides chromatographic methods are widely used. But because of different nature and chemical properties of various systemic fungicides, there is no one method for such studies. Further complexities arise as many of these fungicides, after entering the host plant, breakdown and may often form newer chemical complexes in combination with host constituents which may vary among species and varieties of plants. In such cases residue studies of the fungicide can not be studied properly without developing a suitable and appropriate method.

A method was developed and in a series of experiments by using thin layer chromatography, it was found that in wheat, the fungicide triadimefon, after entering host system changed to another chemical viz., triadimenol. Triadimefon, as such could not be detected in wheat tissues chromatographically. It is thus inferred from the results obtained that by test fungus method and TLC method triadimefon, after entering wheat plant changes to another chemical i.e., triadimenol which is also active in controlling the rust. These findings are in agreement with those of Kramer(1975) and Gasztonyi and Josepovits(1978). Nevertheless, these two methods indicated the presence of residue in straw which could not be traced in grain samples collected from the plants sprayed with triadimefon at different concentrations.

ACKNOWLEDGEMENT

The authors are grateful to Dr. R.M. Singh, Dean and Dr. H.G. Singh, Director(Res.), Sukhadia University, Udaipur for providing facilities. Thanks are due to M/S Bayer India Ltd, Bombay for providing the funds to conduct this research.

摘 要

*Macrophomina phaseolina*菌을 利用하여 살균제 Triadimefon의 밀 품종 Kharchia와 Lal Bahadur에 있어서 수확후의 잔류량을 조사한 결과 살포식물의 밀짚에서는 Triadimefon이 검출되었으나 밀 종자에서는 검출되지 않았다. 잔류량 조사를 위해 개발된 薄層 크로마토그라피에 의하여도 동일한 결과를 얻었다. 기주체내에서 Triadimefon은 Triadimenol로 전환되었다.

LITERATURES CITED

1. Baxi, Kalpna, B.B. Lal Thakore, B.P. Chakravarti and Sneh Mathur. 1982. Absorption, translocation and persistence of triadimefon in wheat and barley. Indian J. Mycol. Pl. Pathol. 12 : 120~121 (Abstr.).
2. Finney, D.J. 1981. Probit Analysis(2nd Edition). Cambridge University Press, London, 283~297.
3. Gasztonyi, M and Gy. Josepovits 1978. Translocation and metabolism of triadimefon in different plant species. Acta Phytopath. Hung. 13 : 403~415.
4. Jain, J.P., B.B. Lal Thakore, G.L. Khandehical, R.B. Singh and Sueh Mathur. Control of wheat rusts by chemicals. Indian. J. Mycol. Pl. Pathol. 10, 11.
5. Kramer, W., K.H. Ouchl and W. Meisser 1975. Chem. Abstr. 82, 156325., Ger. 2. 324. 010.
6. Tafuri, F., M. Patumi, M. Businelli, C. Marucchini 1978. Determination of residue of Vitavax and Plantvax in grains. J. Agri. Food Chem. 26 : 1344~1346.
7. Tripathi, R.K., A. Singh and S.L. Singh 1976. Residue analysis of wheat grains

and straw produced from Vitavax treated seed. Pesticides 10 : 33~34.

8. Waring, R.H., M.S. Wolfe (1975). Distribution of triadimorph and its metabolites in barley plants grown to maturity. Pesticide Science 6 : 169~172.