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1. John, R. L., *Earth Island J., Spl Rep.,* San Francisco, 1998, 7.
2. Teotia, S. P. S. and Teotia, M., *Indian J. Environ. Toxicol.*, 1991, **1**, 1–16.
3. Lee Hitchcox, D. C., Internet Search, Fluoride–Health World Online, 1996.
4. Singh, A., Yadav, G. P. and Gupta, A. P., *Ann. Biol.*, 1990, **6**, 101–104.
5. Singh, V., Gupta, M. K., Rajwanshi, P., Mishra, S., Srivastava, S., Shrivastav, R., Srivastava, M. M., Prakash, S. and Dass, S., *Fd. Chem. Toxic.*, 1995, **33**, 399–402.
6. Staunton, S. and Nye, P. H., *J. Soil Sci.*, 1989, **40**, 751–760.
7. Kage H., *Z. Pflanzenernaehr. Bodenkd*, 1997, **160**, 171–178.
8. Pandeya, S. B., Singh, A. K. and Dhar, P., *Plant Soil*, 1998, **198**, 117–125.
9. Black, C. A., *Methods of Soil Analysis*, Am. Soc. Agron., USA, 1965.
10. APHA, Standard Methods for the Examination of Water and Waste Water, American Public Health Association, Washington DC, 16th edn, 1985.
11. Kemper, W. D., in *Methods of Soil Analysis. Part I. Physical and Mineralogical Methods*, Agronomy Monograph No. 9, Am. Soc. Agron., USA, 1986.
12. Jackson, M. L., *Soil Chemical Analysis*, Prentice Hall of India Pvt, Ltd, New Delhi, 1967, pp. 162–165.
13. Wang, E. J. and Yu, T. R., *Soil Sci.*, 1989, **147**, 35–39; 91–96; 175–178.
14. Perrott, K. W., Smith, B. F. L. and Inkson, R. H. E., *J. Soil Sci.*, 1976, **27**, 58–67.
15. Davison, A. W., *Fluorides: Effects on Vegetation, Animals and Humans*, Paragon Press, Salt Lake City, Utah, 1984, pp. 61–84.
16. Luther, S. M., Poulsen, M. J., Dudas, M. J. and Rutherford, P. M., *Can. J. Soil Sci.*, 1996, **76**, 83–91.
17. Peek, D. C. and Volk, V. V., *Soil Sci. Soc. Am. J.*, 1985, **49**, 583–586.
18. Bar Yosef, B., Afik, I. and Rosenberg, R., *Ecological Studies, 74, Inorganic Contaminants in the Valdose Zone*, Springer Verlag, New York, 1989, pp. 75–85.

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Increasing the efficacy of difluoromethylornithine to inhibit the growth of three phytopathogenic fungi by membrane modifying agents

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The selective inhibition of fungal polyamine biosynthesis using difluoromethylornithine (DFMO) has been a promising approach for plant chemotherapy. However, some plant pathogenic fungi have been found to be resistant to high concentrations of DFMO. Therefore, an attempt was made to increase the efficacy of DFMO to inhibit the growth of three phytopathogenic fungi (*Pythium aphanidermatum*, *Helminthosporium oryzae* and *Rhizoctonia solani*) by using $CaCl_2$ and polyethylene glycol (PEG). The growth inhibition was increased by 12–30% for all the fungi tested with adjuvant concentrations in a selected range (5 mM $CaCl_2$ and 5% PEG), which were otherwise not inhibitory for normal growth of fungi. These results suggest that the effective control of plant pathogenic fungi may be achieved by the use of DFMO in conjunction with $CaCl_2$ or PEG.

POLYAMINES (PAs) are essential for normal growth and development and also in the regulation of several cellular and molecular functions[1–3]. Putrescine (Put), which is

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1. John, R. L., *Earth Island J., Spl Rep.,* San Francisco, 1998, 7.
2. Teotia, S. P. S. and Teotia, M., *Indian J. Environ. Toxicol.*, 1991, **1**, 1–16.
3. Lee Hitchcox, D. C., Internet Search, Fluoride–Health World Online, 1996.
4. Singh, A., Yadav, G. P. and Gupta, A. P., *Ann. Biol.*, 1990, **6**, 101–104.
5. Singh, V., Gupta, M. K., Rajwanshi, P., Mishra, S., Srivastava, S., Shrivastav, R., Srivastava, M. M., Prakash, S. and Dass, S., *Fd. Chem. Toxic.*, 1995, **33**, 399–402.
6. Staunton, S. and Nye, P. H., *J. Soil Sci.*, 1989, **40**, 751–760.
7. Kage H., *Z. Pflanzenernaehr. Bodenkd*, 1997, **160**, 171–178.
8. Pandeya, S. B., Singh, A. K. and Dhar, P., *Plant Soil*, 1998, **198**, 117–125.
9. Black, C. A., *Methods of Soil Analysis*, Am. Soc. Agron., USA, 1965.
10. APHA, Standard Methods for the Examination of Water and Waste Water, American Public Health Association, Washington DC, 16th edn, 1985.
11. Kemper, W. D., in *Methods of Soil Analysis. Part I. Physical and Mineralogical Methods*, Agronomy Monograph No. 9, Am. Soc. Agron., USA, 1986.
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13. Wang, E. J. and Yu, T. R., *Soil Sci.*, 1989, **147**, 35–39; 91–96; 175–178.
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16. Luther, S. M., Poulsen, M. J., Dudas, M. J. and Rutherford, P. M., *Can. J. Soil Sci.*, 1996, **76**, 83–91.
17. Peek, D. C. and Volk, V. V., *Soil Sci. Soc. Am. J.*, 1985, **49**, 583–586.
18. Bar Yosef, B., Afik, I. and Rosenberg, R., *Ecological Studies, 74, Inorganic Contaminants in the Valdose Zone*, Springer Verlag, New York, 1989, pp. 75–85.

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The selective inhibition of fungal polyamine biosynthesis using difluoromethylornithine (DFMO) has been a promising approach for plant chemotherapy. However, some plant pathogenic fungi have been found to be resistant to high concentrations of DFMO. Therefore, an attempt was made to increase the efficacy of DFMO to inhibit the growth of three phytopathogenic fungi (*Pythium aphanidermatum*, *Helminthosporium oryzae* and *Rhizoctonia solani*) by using $CaCl_2$ and polyethylene glycol (PEG). The growth inhibition was increased by 12–30% for all the fungi tested with adjuvant concentrations in a selected range (5 mM $CaCl_2$ and 5% PEG), which were otherwise not inhibitory for normal growth of fungi. These results suggest that the effective control of plant pathogenic fungi may be achieved by the use of DFMO in conjunction with $CaCl_2$ or PEG.

POLYAMINES (PAs) are essential for normal growth and development and also in the regulation of several cellular and molecular functions[1–3]. Putrescine (Put), which is

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the precursor for higher polyamines, spermidine (Spd) and spermine (Spm) is synthesized from arginine and ornithine by the rate-limiting enzymes arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) respectively. Both these pathways for Put synthesis are present in higher plants but most of the fungi possess only the ODC pathway for Put synthesis. The absolute requirement of PAs for growth and development and the presence of a single biosynthetic pathway in many fungi presents an effective chemotherapeutic method for the control of fungal infections in plants[4–6]. A potent substrate-based irreversible inhibitor of ODC enzyme, difluoromethylornithine (DFMO) has been used to selectively inhibit the mycelial growth of several fungi *in vitro*[7–10] and in the control of several important plant diseases, including rust of bean, wheat and *Vicia faba*[4–6,10,11], *Verticillium* wilt of tomatoes[12] and powdery mildew of wheat[5] by targeting Put synthesis enzyme ODC. Interestingly, the use of DFMO did not cause any effect on plant development and PA metabolism as plants possess an alternate biosynthetic pathway, i.e. ADC which is more predominant than ODC[13,14]. However, some fungi have been found to be highly resistant to DFMO and therefore, not amenable to growth inhibition with lower concentrations of DFMO[3,6,15–18]. This apparent resistance of some fungi could be due to various reasons like problems in the uptake of DFMO, degradation of DFMO in the cell, higher endogenous PA levels and ODC activity in the fungal cells[19], resistance of the target enzyme ODC to DFMO or the presence of an alternate pathway for PA biosynthesis[20,21]. To gather some clues underlying this differential response as well as to overcome the resistance of fungi to DFMO, various membrane-modifying compounds were used as adjuvants along with DFMO. This was based on the presumption that uptake of DFMO by the fungi is the critical factor. Previously too, some amino acid-based inhibitors of ODC have been reported to cause increased inhibition than that caused by DFMO due to their greater permeability[22]. The fungi included in the present study were categorized as highly susceptible (*Rhizoctonia solani*), moderately susceptible (*Helminthosporium oryzae*) and highly resistant (*Pythium aphanidermatum*) to DFMO[10,20].

The cultures of *R. solani* (accession no. 2775) and *H. oryzae* (accession no. 2785) were obtained from the Division of Mycology, Indian Agricultural Research Institute, New Delhi. *P. aphanidermatum* cultures were already available with the Department of Microbiology, University of Delhi, South Campus, New Delhi.

The fungi were grown on Czapek-dox-agar medium along with different concentrations of the adjuvants [$CaCl_2$, polyethylene glycol (PEG 6000–7500 obtained from SRL, India), Tween-20, ascorbic acid, dimethylsulphoxide (DMSO) and α-tocopheryl acetate] in dark at 26 ± 1°C to select for the range of concentrations of adjuvant, which did not affect the normal fungal growth. A sterile cork borer was used to cut a mycelial plug of 7 mm diameter from the growing periphery of the fungal mat and it was placed upside down in the centre of a 90 mm petri dish containing Czapek-dox-agar medium with and without the various supplements to allow for a uniform radial growth of the fungi. The colony diameter was recorded after 1, 2 and 3 days of culture for *R. solani*, after 2, 4 and 6 days for *P. aphanidermatum* and for *H. oryzae* after 3, 6 and 9 days of culture. The 7 mm plug which was inoculated was not included in the measurement of the colony diameter[20].

The adjuvant concentrations selected were 1, 3 and 5 mM for $CaCl_2$, 3 and 5% for PEG, 1, 5 and 10 mg/l for ascorbic acid, 0.001% for Tween-20, 0.1% for DMSO and 0.1% for α-tocopheryl acetate. These adjuvant concentrations which did not affect the fungal growth were then considered along with DFMO concentration which caused 20–40% growth inhibition in a control culture. The DFMO concentrations used were 0.1 and 0.5 mM for *R. solani*, 1 mM for *H. oryzae* and 10 mM for *P. aphanidermatum*[10,20]. Fungal growth inhibition was compared on a medium containing DFMO alone or DFMO in conjunction with the adjuvant with the growth on the medium having no inhibitor (but with or without the adjuvant).

Of the various adjuvants studied, only $CaCl_2$ and PEG were found to augment the antifungal activity of DFMO against three plant pathogenic fungi, *R. solani*, *H. oryza*, *P. aphanidermatum* and some of the results are shown in Figures 1–3. The growth of *R. solani* was inhibited by 40% in the presence of 0.5 mM DFMO. Its growth was further inhibited in the presence of either $CaCl_2$ (3 and 5 mM) or PEG (3 and 5%) along with DFMO to 43%. Lower concentrations of $CaCl_2$ (1 mM) and PEG (1%) were not found to be effective in increasing the inhibition,

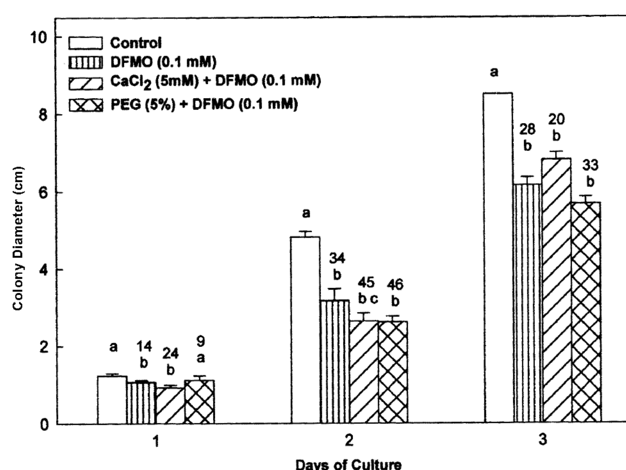

Figure 1. Colony diameter of *R. solani* in the presence of DFMO (0.1 mM) alone and in combination with $CaCl_2$ (5 mM) and PEG (5%). The vertical bars represent the mean ± SEM. The bars with different letters have significantly different means ($P \leq 0.05$) using Fischer's LSD procedure. The numbers on the bars are the per cent inhibition values compared to the mycelial growth in control cultures.

and concentrations of adjuvants higher than those selected affected the normal fungal growth. DMSO, α-tocopheryl acetate and Tween-20, all affected the normal growth of *R. solani*. In case of $CaCl_2$ and PEG, 5 mM $CaCl_2$ and 5% PEG were found to be the most effective and the cultures with inhibited growth had sparse hyphae as the growth of these cultures was drastically reduced by DFMO.

A significant increase in the efficacy of DFMO was caused by $CaCl_2$ and PEG along with 0.1 mM DFMO which otherwise caused 30% inhibition in the growth of *R. solani*. In the presence of 5 mM $CaCl_2$ and 5% PEG, 0.1 mM DFMO caused 45% and 46% inhibition respectively (Figure 1).

The growth of *P. aphanidermatum* was inhibited by 20% in presence of 10 mM DFMO but in the presence of 5 mM $CaCl_2$ inhibition was increased to 39% and to 51% in presence of 5% PEG (Figure 2). Growth inhibition in the presence of 1 mM $CaCl_2$ and 3% PEG was not found to be as effective as the above-mentioned concentrations. Tween-20 at 0.001% was also able to increase the inhibition to 32%. DMSO (0.1%) and α-tocopheryl acetate (0.1%) were not able to increase the inhibition caused by DFMO.

Ascorbic acid (1, 3 and 5 mg/l) did not enhance the inhibition caused by DFMO in *R. solani* at a DFMO concentration of 0.1 mM, but it caused an increase in the growth inhibition of *P. aphanidermatum* to 26%. A significant increase in the growth rate of the fungi was observed in the presence of ascorbic acid and the fungi sporulated earlier than the control cultures on the medium without any ascorbic acid, this may be attributed to the fact that vitamins are essential for growth and are readily taken up by the cells, leading to increased growth rate, hence ascorbic acid was not used for further experiments.

For *H. oryzae* 1 mM DFMO was considered and it caused 20% inhibition of growth in control cultures but in presence of 5 mM $CaCl_2$ and 5% PEG the inhibition was increased to 25% and 29% respectively (Figure 3).

The adjuvants that have been chosen for the present study are basically membrane-modifying agents ($CaCl_2$, PEG, Tween-20, DMSO), other than ascorbic acid and α-tocopheryl acetate, both of which are antioxidants. Since the differential uptake of DFMO by different fungi has been previously observed and is correlated to the degree of inhibition[17,18], a membrane-modifying agent might increase the uptake of DFMO by causing some perturbations in the membrane and increase the inhibition, also these compounds are chemically stable and reasonably non-toxic at the concentrations selected for augmenting the DFMO effect. DMSO and Tween-20 were not found to be helpful for *R. solani*, however, Tween-20 was effective in case of *P. aphanidermatum* and therefore very low concentrations of Tween-20 were used for the other fungi but it did not augment the inhibition. The ineffectiveness of Tween-20 and DMSO may be due to their poor effect on the modification of the membrane at the concentrations selected and hence there being no effective increase in the uptake of DFMO, at higher concentrations these adjuvants proved detrimental for the fungal growth. PEG and $CaCl_2$ were found to be most effective probably due to their miscibility in the medium, leading to even distribution and also as these chemicals do not interfere with any other cellular function other than perturbing the membrane to an extent at the concentrations selected, therefore these could be used at concentrations which were reasonably high to be able to increase the uptake of DFMO. Therefore a balance has to be maintained between the adjuvant concentration which does not affect the

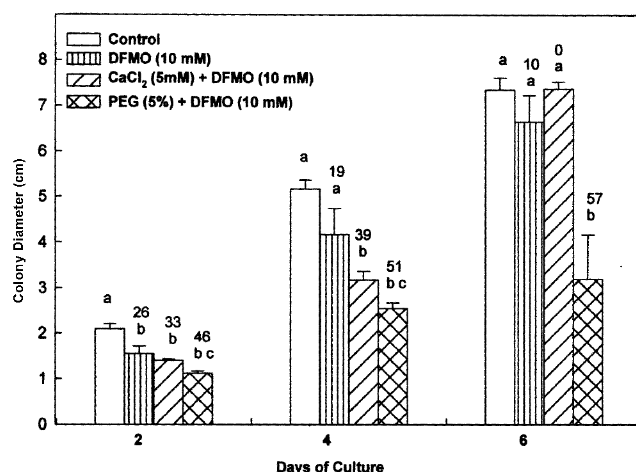

Figure 2. Colony diameter of *P. aphanidermatum* in the presence of DFMO (10 mM) alone and in combination with $CaCl_2$ (5 mM) and PEG (5%). The vertical bars represent the mean ± SEM. The bars with different letters have significantly different means ($P \leq 0.05$) using Fischer's LSD procedure. The numbers on the bars are the per cent inhibition values compared to the mycelial growth in control cultures.

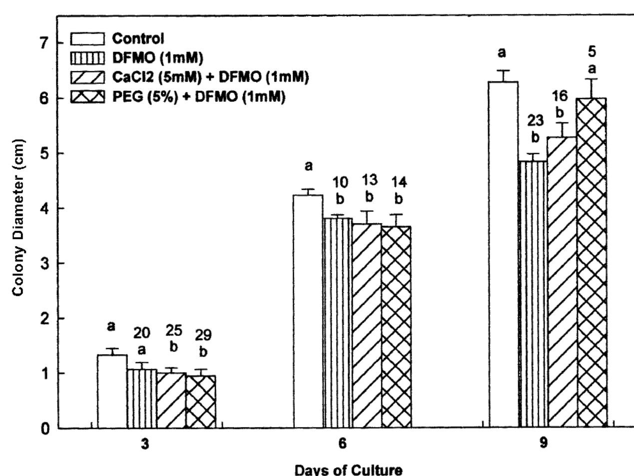

Figure 3. Colony diameter of *H. oryzae* in the presence of DFMO (1 mM) alone and in combination with $CaCl_2$ (5 mM) and PEG (5%). The vertical bars represent the mean ± SEM. The bars with different letters have significantly different means ($P \leq 0.05$) using Fischer's LSD procedure. The numbers on the bars are the per cent inhibition values compared to the mycelial growth in control cultures.

normal fungal growth and yet enhances the effect of DFMO.

The differential effect of the adjuvants on increasing the efficacy of DFMO could also be due to the diversity of fungal cell wall chemistry, thus varying the actual concentration of adjuvant in the vicinity of the membrane[23] and the susceptibility of the fungal ODC to DFMO inhibition.

All the three fungi showed some recovery of the fungal growth over a period of time, though the recovery was slow in case of fungi growing on adjuvant-supplemented medium, except *H. oryzae* which showed faster recovery on the adjuvant plus DFMO medium than the DFMO medium. This may be because of the inherent properties of the fungal cell membrane, such that it could overcome the perturbations caused by the adjuvants faster than the other fungi. The mycelial plug taken from a culture of *R. solani* which showed partial recovery on the DFMO (0.1 mM) supplemented medium, when inoculated on control medium, showed complete recovery of growth. On the other hand, on a medium containing DFMO (0.1 mM), the recovering fungi was inhibited to 48% instead of 20% and in presence of 5% PEG along with DFMO (0.1 mM) no growth was observed for 2 days of culture and only few hyphae appeared on the 3rd day.

The partial recovery of the fungi might be due to the gradual acclimatization of the fungi to the medium or the DFMO concentrations in the medium might become limiting[20]. Since no fungal growth was observed for the first two days of the repeat culture on the DFMO-supplemented medium, the complete inhibition of the fungal growth might be achieved by the repeated use of DFMO along with a suitable adjuvant, this holds a lot of significance in the chemotherapeutic application of DFMO. Moreover, DFMO does not affect the normal plant growth and development, PA metabolism and chromosome behaviour even at very high concentrations, i.e. 5 mM (refs 13, 14, 24). Further, DFMO also triggers the defence mechanisms of the host plants[25,26].

The variation in pH of the medium in the range of 5.5 to 7.0 in an attempt to enhance the inhibition caused by DFMO demonstrated that a pH lower than 5.5 and higher than 7.0 was not conducive for normal fungal growth, though greater inhibition of growth of *R. solani* was observed with 0.1 mM DFMO when the pH of the medium was 5.5. Hence we conclude that the efficacy of DFMO can be much enhanced in the presence of certain adjuvants which increase the uptake of DFMO. Therefore the use of these adjuvants along with DFMO, which is a non-phytotoxic compound[14] can be effectively applied for the control of fungal plant infections.

1. Galston, A. W., *Bioscience*, 1983, **33**, 102–109.
2. Smith, T. A., *Annu. Rev. Plant Physiol.*, 1985, **36**, 117–143.
3. Rajam, M. V., in *Plant Ecophysiology* (ed. Prasad, M. N. V.), John Wiley, New York, 1997, pp. 343–374.
4. Rajam, M. V., Weinstein, L. H. and Galston, A. W., *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 6874–6878.
5. Weinstein, L. H., Osmeloski, J. F., Wettlaufer, S. H. and Galston, A. W., *Plant Sci.*, 1987, **51**, 311–316.
6. Rajam, M. V., *Curr. Sci.*, 1993, **65**, 461–469.
7. West, H. M. and Walters, D. R., *Mycol. Res.*, 1989, **92**, 453–457.
8. Foster, S. A. and Walters, D. R., *J. Gen. Microbiol.*, 1990, **136**, 233–239.
9. Sinha, M. and Rajam, M. V., *Indian J. Exp. Biol.*, 1992, **30**, 538–540.
10. Bharti and Rajam, M. V., *Mycopathologia*, 1996, **133**, 95–103.
11. Walters, D. R., *New Phytol.*, 1986, **104**, 613–619.
12. Mussell, H., Osmeloski, J., Wettlaufer, S. H. and Weinstein, L. H., *Plant Dis.*, 1987, **71**, 313–316.
13. Rajam, M. V., Weinstein, L. H. and Galston, A. W., *Curr. Sci.*, 1991, **60**, 178–180.
14. Bharti and Rajam, M. V., *Ann. Bot.*, 1995, **76**, 297–301.
15. West, H. M. and Walters, D. R., *Mycol. Res.*, 1991, **95**, 621–623.
16. Havis, N. D. and Walters, D. R., *Lett. Appl. Microbiol.*, 1992, **14**, 244–246.
17. Smith, T. A., Barker, J. H. A. and Owen, W. J., *Mycol. Res.*, 1992, **5**, 395–400.
18. Walters, D. R., Keenan, J. P., Cowley, T., McPherson, A. and Havis, N. D., *Plant Pathol.*, 1995, **44**, 80–85.
19. Barker, J. H. A., Smith, T. A. and Owen, W. J., *Mycol. Res.*, 1993, **11**, 1358–1362.
20. Rajam, M. V. and Galston, A. W., *Plant Cell Physiol.*, 1985, **26**, 683–692.
21. Khan, A. J. and Minocha, S. C., *Life Sci.*, 1989, **44**, 1215–1222.
22. Smith, T. A., Barker, J. H. A. and Jung, M., *J. Gen. Microbiol.*, 1990, **136**, 985–992.
23. Bartnicki-Garcia, S., *Annu. Rev. Microbiol.*, 1968, **22**, 87–108.
24. Zarb, J. and Walters, D. R., *New Phytol.*, 1994, **126**, 99–104.
25. Bharti, Rajam, M. V. and Sawhney, R. N., *Phytochemistry*, 1996, **43**, 1009–1013.
26. Rajam, M. V. and Bharti, in *Microbes: For Health, Wealth and Sustainable Environment* (ed. Verma, A.), Malhotra Publishing House, New Delhi, 1998, pp. 139–157.

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