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ORIGINAL ARTICLE/RESIDUE AND SAFETY

Influence of Hexaconazole on Biochemical Constituents of Groundnut

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Abstract

In this study, hexaconazole 5% SC, an ergosterol biosynthesis inhibitor, was tested on groundnut with its recommended (500 mL ha^{-1}) and higher (2,000 mL ha^{-1}) concentrations under greenhouse conditions in India. Its influence on biochemical constituents of groundnut plants was assessed apart from its disease management potential against late leaf spot caused by *Phaeoisariopsis personata* (Berk and Curt). Likewise, leaf samples were collected from hexaconazole 5% SC-sprayed plants at different time intervals. Thereafter, their analyses showed considerable differences in the plant constituents, such as chlorophyll, soluble protein, and total phenol contents and the activity of nitrate reductase enzyme. The induction activity of defense-related enzyme, peroxidase, was also analyzed. However, no difference was observed in the isozymic pattern. Moreover, the ground kernels collected from treated plants also showed no difference in the estimated carbohydrate and other constituents.

Key words groundnut, hexaconazole, biochemical constituents, peroxidase

Introduction

The groundnut's late leaf spot is caused by *Phaeoisariopsis personata* (Berk and Curt). V. Arx causes a yield loss of up to 53% (Patel and Vaishnav, 1987). Given that the late leaf spot pathogen is seed-borne and soil-borne (Suryanarayana, 1978), it reduces the loss regardless of the transmission method. Hexaconazole, a broad spectrum fungicide of the triazole group, was synthesized at the Jealott's Hill Research Station at the plant protection division of the ICI Agrochemicals (Shephard *et al.*, 1986). It is being sold under the brand names Anvil and Planette (Smith, 1991). Basically it inhibits the ergosterol biosynthesis, the principal sterol in the membranes of most fungi (Sisler *et al.*, 1983). It is recommended to be used at the rate of 25 to 37.5 g a.i. ha^{-1} (500 to 750 mL commercial product ha^{-1}) for the management of various ascomycetes and basidiomycetes (Worthington, 1991). The bioefficacy of the triazole compound hexaconazole has been reported by several researchers (Subrahmanyam *et al.*, 1990; Jadeja *et al.*, 1999; Ali and Archer, 2003). Apart from being fungitoxic, EBIs (ergosterol biosynthesis inhibitors) alter the plant in variety of ways (Fletcher *et al.*, 2000). This leads to a variety of additional advantages such as growth promotion, increase in yield, quantity, and quality of the products (Baby *et al.*, 2004). Increased chlorophyll content caused by triazole chemicals (Fletcher *et al.*, 1986; Lee *et al.*, 1999; Singh and Thakore, 1998) resulted in the following advantages: increased carbohydrates by paclobutrazol (Lambers, 1982), and increased accumulation of total phenol by carbendazim application (Singh and Kang, 1978; Sharma *et al.*, 1990), all of which were well

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documented.

Chemicals including fungicides and herbicides have also been reported to act as inducers for systemic resistance in plant systems against pathogens. Likewise, it also reduces the severity of some diseases (Davidse and Ward, 1984). Oostendrop *et al.* (1996) reported that triazole fungicides such as epoxiconazole and propiconazole induced systemic resistance against *Colletotrichum lagenarium* in cucumber. Moreover, the probenazole treatment for *Xanthomonas oryzae* pv. *oryzae* management resulted in the accumulation of pathogenesis-related protein (PR protein), heat shock protein and thylakoid protein in rice leaves (Lalithakumari and Dhakshinamurthy, 1995). Different isoforms of chitinases were purified from pepper stems treated with mercuric chloride (Kim and Hwang, 1996). Hence, the present study was conducted to investigate the effect of hexaconazole 5% SC on the biochemical constituents of groundnut apart from its disease managing potential.

Materials and Methods

To determine the effect of different fungicides on groundnut leaves, experiments were conducted under glasshouse conditions. Seeds of groundnut cv. Co2 were sown at the rate of 5 seeds/pot. Three replications were arranged following completely randomized block design. Fungal spores were collected from infected leaves. In addition, the conidial suspension (5×10^4 spores) was sprayed on 35 days old plants using an atomizer. On day 45, the plants were sprayed with two different concentrations of hexaconazole 500 and 2,000 mL ha^{-1}, carbendazim 500 g ha^{-1}, mancozeb 1 kg ha^{-1}, chlorothalonil 1 kg ha^{-1} and distilled water (uninoculated control). Moreover, subsequent sprays were done thrice at 15-day intervals. Leaf samples were collected at 0, 1, 3, and 5 days after the last spray from each treatment and used for analyses of chlorophyll, nitrate reductase, protein, and phenol contents.

Estimation of plant components

Chlorophyll a, b, and total chlorophyll contents were estimated through the procedure developed by Willey *et al.* (1999) from 500 mg of groundnut leaf samples. The 80% acetone alone was used as a check and measured the observance at 645, 663, and 652 nm for chlorophyll a, b, and total chlorophyll contents, respectively. Furthermore, they were expressed as mg g^{-1} of fresh weight. The nitrate reductase activity was measured as described by Nicholas *et al.* (1976) from 500 mg of groundnut leaf samples and expressed as μ moles of nitrite-produced h^{-1} g^{-1} of fresh weight. Soluble protein and total phenol contents were estimated following the methods of Lowry *et al.* (1951) and Bray and Thorpe (1954), respectively. Moreover, they were expressed in terms of mg g^{-1} of fresh tissue. Total carbohydrates in the harvested groundnut kernels were estimated by following the method of Hedge and Hofreiter (1962) and expressed as percentage of fresh weight.

Peroxidase activity

The plants were inoculated with the pathogen *P. personata* two days after the spraying of the fungicide. Thereafter, the samples were collected on 0, 1, 2, 3, and 5 days after inoculation. The samples were also collected on respective days from the fungicide sprayed-uninoculated plants. Subsequently, the peroxidase activity was estimated by following Hammerschmidt *et al.* (1982) and expressed as change in absorbance at 470 nm min^{-1} g^{-1} fresh tissue.

Native anionic polyacrylamide gel electrophoresis, resolving gel of 8% acrylamide, and stacking gel of 4% acrylamide were prepared and loaded with the enzyme extract. Electrophoresis was carried out at constant voltage of 65 V in cold condition. After the electrophoresis, the gels were stained with the staining solution containing 0.05% benzidine and 0.03% hydrogen peroxide in 0.02 M acetate buffer (pH 4.5). After staining for 3 min, the gel was immersed in 7% acetic acid for 3 min and subsequently washed with distilled water (Nadlony and Sequira, 1980).

Results

The chlorophyll *a* content ranged from 1.52 to 1.78 mg g^{-1} and chlorophyll *b* ranged from 0.45 to 0.58 mg g^{-1} on day 3 and 5 from hexaconazole spray, while all the three forms of chlorophyll were at the lowest (0.84, 0.28, and 1.12 mg g^{-1}, respectively) in the *P. personata* inoculated groundnut plants on day 5 (Table 1). Hexaconazole 500 mL ha^{-1} (2.12 mg g^{-1}) and carbendazim (2.06 mg g^{-1}), and macozeb (1.83 mg g^{-1}) have also increased the total chlorophyll content compared to uninoculated control plants (1.73 mg g^{-1}) on day 5 after spraying.

The nitrate reductase activity increased to 3.22 and 3.36 μM of NO_2 produced g^{-1} h^{-1} on day 5 by spraying hexaconazole at doses of 500 and 2,000 mL ha^{-1}, respectively. The minimum enzyme activity was found in the plants inoculated with *P. personata* alone (2.76 μmole of NO_2 g^{-1} hr^{-1}) on day 3. All the fungicide treatments were superior to the inoculated and uninoculated-unsprayed control in increasing the nitrate reductase activity (Fig. 1).

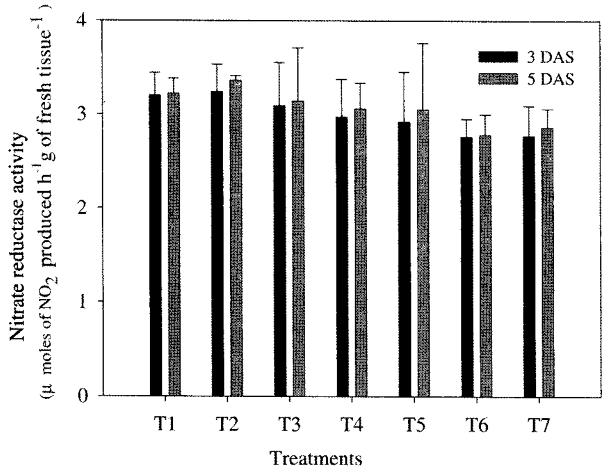

Fig. 1. Effect of fungicide treatment and inoculation of *Phaeoisariopsis personata* on nitrate reductase activity of groundnut leaves. Bars indicate the mean of three replicates + standard error. DAS: days after spraying of fungicides, T1: hexaconazole 5% SC 500 mL ha^{-1}, T2: hexaconazole 5% SC 2000 mL ha^{-1}, T3: carbendazim 500 g ha^{-1}, T4: mancozeb 1,000 g ha^{-1}, T5: chlorothalonil 1,000 g ha^{-1}, T6: pathogen inoculation only, T7: no treatment control.

Similarly, the soluble protein content also increased from 74.58 and 78.63 mg g^{-1} on the third day to 77.61 and 80.36 mg g^{-1} on the fifth day after the hexaconazole spray at a rate of 500 and 2,000 mL ha^{-1}, respectively. Likewise, it was significantly different from other treatments. The protein content was low in the inoculated plants (41.16 mg g^{-1}) on day 5 where the protein content decreased with increase in days after inoculation. The maximum amount of total phenol was recorded in hexaconazole at 2,000 mL ha^{-1} (11.87 mg g^{-1}) treatment on day 5 followed by a low dose (10.72 mg g^{-1}) and carbendazim (8.94 mg g^{-1}). Among the fungicide treatments, the chlorothalonil recorded the lowest phenol content of 8.66 mg g^{-1} (Table 2). However, all these treatments had no influence on the carbohydrate, protein, and oil contents in groundnut kernels (data not shown).

Peroxidase activity in groundnut

The change in peroxidase activity was observed between the treatments in each day after spraying. Significant induction was detected on day 2 from spraying. Thereafter, it was maintained at the same level up to day 3

Table 1. Effect of fungicides on chlorophyll content of groundnut leaves

Treatment	Dosage	Chlorophyll content (mg g^{-1} leaf)					
		3 DAS [a]			5 DAS [a]		
		Chlorophyll a	Chlorophyll b	Total chlorophyll	Chlorophyll a	Chlorophyll b	Total chlorophyll
Hexaconazole 5% SC	500 mL ha^{-1}	1.52 b	0.45 b	1.97 b	1.64 b	0.48 b	2.12 b
Hexaconazole 5% SC	2000 mL ha^{-1}	1.59 a	0.49 a	2.08 a	1.78 a	0.58 a	2.36 a
Carbendazim	500 g ha^{-1}	1.40 c	0.42 bc	1.83 c	1.55 c	0.51 b	2.06 c
Mancozeb	1000 g ha^{-1}	1.29 d	0.37 de	1.67 d	1.40 d	0.43 c	1.83 d
Chlorothalonil	1000 g ha^{-1}	1.21 e	0.34 e	1.55 e	1.25 f	0.39 d	1.64 f
Control 1	Inoculated	1.02 f	0.30 f	1.32 f	0.84 g	0.28 e	1.12 g
Control 2	No treatment	1.29 d	0.40 cd	1.69 d	1.31 e	0.42 cd	1.73 e

Values in each column are the mean of three replicates. Each column values followed by a same letter are not significantly different at the 5% level by DMRT. [a] Days after spraying.

Table 2. Effect of fungicides on soluble protein and phenol contents of groundnut leaves

Treatment	Dosage	Soluble protein (mg g^{-1} fresh tissue)		Total phenol (mg g^{-1} fresh tissue)	
		3 DAS [a]	5 DAS [a]	3 DAS [a]	5 DAS [a]
Hexaconazole 5% SC	500 mL ha^{-1}	74.58 b	77.61 b	8.69 b	10.72 b
Hexaconazole 5% SC	2000 mL ha^{-1}	78.63 a	80.36 a	9.50 a	11.87 a
Carbendazim	500 g ha^{-1}	66.64 c	75.43 c	7.85 c	8.94 c
Mancozeb	1000 g ha^{-1}	54.64 d	68.96 d	7.50 d	8.79 d
Chlorothalonil	1000 g ha^{-1}	50.26 f	66.33 e	6.39 e	8.66 e
Control 1	Pathogen inoculated	44.34 g	41.16 g	5.99 f	8.34 f
Control 2	No treatment	52.74 e	53.16 f	5.10 g	5.34 g

Values in each column are the mean of three replications. Each column values followed by a same letter are not significantly different at the 5% level by DMRT. [a] Days after spraying.

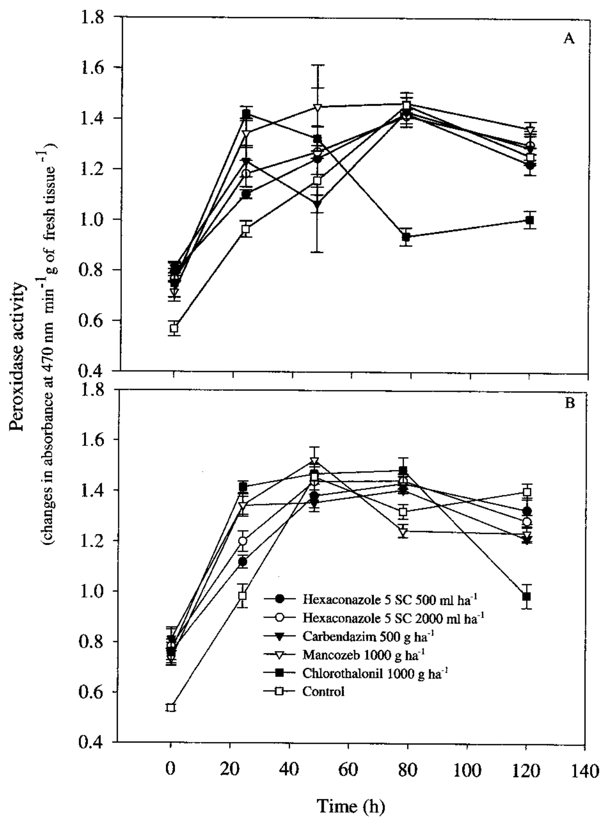

Fig. 2. Induction of peroxidase activity in groundnut leaves treated with fungicides (A: uninoculated, B: inoculated with *Phaeoisariopsis personata*). Values are the mean of three replicates ± standard error.

after spraying. This was maintained until the activity declined. Even though the induction of peroxidase activity was observed in hexaconazole treatment, the enzyme activity was not at an appreciable amount (Fig. 2).

Native gel electrophoretic separation of crude peroxidase enzyme extract from groundnut showed a weak induction of four isoforms of peroxidase. However, the induction was uniformly found in all the treatments (data not shown).

Discussion

Carbendazim was reported to increase the chlorophyll *a*, *b*, total chlorophyll, and total phenol content in groundnut plants (Singh and Kang, 1978) and chilli plants (Sharma *et al.*, 1990). In this research, hexaconazole 5% SC resulted in a better increase of those constituents than carbendazim did. Increased phenol and carbohydrate contents caused by various fungicides were reported to reduce the disease incidence in wheat plants (Sindhan *et al.*, 1996) and in chick pea owing to bavistin and vitavax treatments (Singh and Sindhan, 1998). Moreover, it similarly increased the chlorophyll contents in bottle gourd (Singh and Thakore, 1998) and cucumber (Lee *et al.*, 1999) owing to hexaconazole spray; changes in the biometric and physiological parameters of tea owing to EBIs spray on tea (Baby *et al.*, 2004) were also reported.

The presence of more phenolic compounds is always correlated with disease resistance. The downy mildew and white rust resistant and moderately resistant cultivars of *Brassica juncea* contained higher amount of chlorophyll (*a*, *b*, and total), sugars, and total phenols than the susceptible cultivar of *B. juncea* (cv. Varuna) (Singh, 2000). Similarly, Bhatia *et al.* (1972) observed that phenol content was higher in resistant varieties than in susceptible varieties of tomato infected with early blight (*Alternaria alternata*) disease. The increased phenol content observed in the present study may also be responsible for disease resistance. Srivastava (1980) reported that the activity of nitrate reductase in plants gives a good estimate of nitrogen contents of plants and often correlated with growth and yield. Kotastane and Vyas (1992) also reported a similar result in mustard after carbendazim, mancozeb, and zineb application as foliar spray; seed treatment; and soil drenching. In this study, the increased nitrate reductase activity was found as well (Fig. 1). It is possible that this also might improve the nitrogen cntents, thus resulting in increased growth, vigor, and yield of groundnut. The increased carbohydrate, soluble protein, and nitrate reductase contents in groundnut attributed to the hexaconazole spray will ultimately lead to improved plant vigor and potentially restrict the development of *Phaeoisariopsis personata*.

Chemical fungicides often act as inducers of defense enzymes in plants against pathogens. Davidse and Ward (1984) reported that the systemic fungicide probenazole treatment induced and accumulated many defense enzymes including peroxidase in the treated leaves. They concluded that the disease controlling effect of probenazole was attributed to a host-mediated reaction. The present study also revealed the changes in peroxidase activity from day 1 to day 3 after spraying. This might have contributed to the defense response against the establishment of pathogen.

Fletcher *et al.* (2000) stated that the effect of triazole on plants may be inhibitory or stimulatory depending on the compound, concentration used, and the type of plant. In this study, it was found that the fungicide influenced the biochemical constituents of groundnut. Likewise, it was found that the influence may lead to improved physiological functions. Apart from the ergosterol biosynthesis inhibition, the metabolic changes leading to unfavorable environment for *P. personata* growth and disease development possibly contributed to the enhanced efficacy of hexaconazole against the onset of groundnut late leaf spot disease.

Acknowledgement

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Literature Cited

Ali, M. and S.A. Archer (2003) Evaluation of some new fungicides against sheath blight disease of rice caused by *Rhizoctonia solani*. Bangladesh J. Pl. Pathol. 19:13~20.

Baby, U. I., S. Balasubramanian, D. Ajay and R. Premkumar (2004) Effect of ergosterol biosynthesis inhibitors on blister blight disease, the tea plant and quality of made tea. Crop Protection 23:795~800.

Bhatia, I. S., D. S. Uppal and K. L. Bajaj (1972) Studies on phenolic contents of resistant and susceptible varieties of tomato (*Lycopersicon esculentum*. L) in relation to early blight. Indian Phytopath. 25:231~235.

Bray, H. G. and W. V. Thorpe (1954) Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal. 1:27~52.

Davidse, L. C. and M. A. de. Ward (1984) Systemic fungicides. In: *Advances in Plant Pathology*. Vol. 2. Academic press. London. pp. 191~257.

Fletcher, R. A., G. Hofstra and J. Gao (1986) Comparative fungitoxic and plant growth regulating properties of triazole derivatives. Plant Cell Physiol. 27:367~371.

Fletcher, R. A., A. Gilley, N. Sankhla and T. D. Davis (2000) Triazoles as plant growth regulators and stress protectants. Hortic. Rev. 24:55~138.

Hammerschmidt, R., E. M. Nuckles and J. Kuc (1982) Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol. 20:73~82.

Hedge, J. E. and B. T. Hofreiter (1962) In: *Carbohydrate chemistry*. (Eds.) R. L. Whistler and J. N. Bemiller, Academic press, New York. Volume 17. p. 420.

Jadeja, K. B., D. M. Nandolia, I. V. Dhruj and R. R. Khander (1999) Efficacy of four triazole fungicides in the control of leaf spots and rust of groundnut. Indian Phytopath. 52:421~422.

Kim, Y. J. and B. K. Hwang (1996) Purification, N-terminal aminoacid sequencing antifungal activity of chitinases from pepper stems treated with mercuric chloride. Physiol. Mol. Plant Pathol. 48:417~432.

Kotastane, A. S. and S. C. Vyas (1992) Phenol contents as influenced by fungicides and by different mode of application on mustard plants. Indian Phytopath. Suppl. 44:Abst.

No. 21.

Lalithakumari, D. and M. Dhakshinamurthy (1995) Control of bacterial leaf blight of paddy by probenazole treatment. Indian J. Mycol. Plant Pathol. 25:118~119.

Lambers, H. (1982) Cyanide-resistant respiration. A non phosphorylating electron transport pathway as an energy overflow. Physiol. Plant. 55:478~485.

Lee, J. M., E. J. Bae, C. W. Lee and S. S. Kwon (1999) Seedling growth and fruit set and quality of cucumber as affected by triazole chemicals. In: *National symposium on quality of fruits and fermented vegetables* (Eds.) J. M. Lee, K. C. Cross, A. E. Watada and S. K. Lee. ISHS, Korea, 1999. pp. 125~132.

Lowry, O. H., N. H. Rosenbrough, A. L. Farr and R. J. Randall (1951) Protein measurement with Folin-phenol reagent. J. Biol. Chem. 193:265~275.

Nadlony, L. and I. Sequira (1980) Increase in peroxidase activities are not directly involved in induced resistance in tobacco. Physiol. Plant Pathol. 16:1~8.

Nicholas, J. C., J. E. Harper and R. H. Hageman (1976) Nitrate reductase activity in soybeans. I. Effect of light and temperature. Plant Physiol. 58:731~735.

Oostendrop, M., H. van Kessman, L. Friedrich, A. Geissmann, J. Gorleach, G. Hengy, R. Nordmeyer, R. Reist, W. Rucss and T. Staub (1996) Influence of plant activator Bion (R) and of triazole fungicides on plant defense mechanisms. Gesunde Pflanzen. 48:260~264.

Patel, V. A. and M. V. Vaishnav (1987) Assessment of losses in groundnut due to rust and tikka leaf spots in Gujarat. Research Journal, Gujarat Agric. Univ. 12:52~53.

Sharma, S. S., M. P. Sharma and G. Rajamani (1990) Effect of carbendazim on chlorophyll, total phenols and mineral content in chilli plants (Capsicum annuum L.) Indian J. Mycol. Plant Pathol. 20:229~233.

Shephard, M. C., R. A. Noon, P. A. Worthington, W. D. McClellan and B. G. Lever (1986) Hexaconazole - a novel triazole fungicide. In: *British crop protection Conference-Pests and diseases*. Vol. 1. pp. 19~26.

Sindhan, G. S., R. D. Parashar and I. Hooda (1996) Effect of chemical seed treatment on incidence of flag smut and biochemical constituents of wheat plants. Indian J. Mycol. Plant Pathol. 26:268~291.

Singh, H. V. (2000) Biochemical basis of resistance in *Brassica* species against downy mildew and white rust of mustard. Pl. Dis. Res. 15:75~77.

Singh, P. and M. S. Kang (1978) Physiological response of groundnut to bavistin application for the control of tikka disease. In: *Symposium on Plant Disease Problems, Oct. 1-3, 1978*, Udaipur. pp. 13~14.

Singh, R. and B. B. L. Thakore (1998) Effect of hexaconazole on chlorophyll and NPK contents of bottlegourd leaves. Indian J. Mycol. Plant Pathol. 28:216~217.

Singh, R. and G. S. Sindhan (1998) Effect of fungicides on the incidence of dry root rot and biochemical status of chickpea plants. Pl. Dis. Res. 13:14~17.

Sisler, H. D., R. C. Walsh and B. N. Ziogas (1983) Ergosterol biosynthesis: a target of fungitoxic action. In: *IUPAC Pesti. Chem. Human Welfare and the Environment* (Eds.) J. Miyamoto and P.C. Kearny, Pergamon Press, New York, pp. 129~134.

Smith, D. B. (1991) Lumber protection in the 90's. *Proceedings of a meeting held at Forintek Canada Corp*, Western Laboratory, compiled by T. Byrne. pp. 53~59.

Srivastava, H. S. (1980) Regulation of nitrate reductase activity in higher plants. Phytochemistry 19:725~733.

Subrahmanyam, P., P. V. S. Rao and D. McDonald (1990) Comparative efficacy of four fungicides against rust and late leaf spot of groundnut. International Arachis News letter 7:21~23.

Suryanarayana, D. (1978) Seed Pathology. Vikas publishing House Pvt., New Delhi. pp. 25~60.

Willey, J. D., H. W. Paerl and M. Go (1999) Impact of rainwater hydrogen peroxide on chlorophyll a content of surface Gulf Stream seawater off North Carolina, USA. Mar. Eco. Prog. ser. 178:145~150.

Worthington, P. A. (1991) 1,2,4-triazole compounds related to the fungicides flutriafol and hexaconazole. Pestic. Sci. 31:457~498.

땅콩의 생화학성분에 미치는 Hexaconazole의 영향

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요 약 인도에서 온실조건 하에 땅콩을 재배하면서 ergosterol 생합성 저해제인 hexaconazole 5% SC를 추천농도(500 mL ha^{-1}) 및 고농도(2,000 mL ha^{-1})로 처리하고 그 영향을 관찰하였다. *Phaeoisariopsis personata*(Berk and Curt)에 의한 점무늬병 방제능력과는 별도로 땅콩식물체의 생화학적 구성성분에 대한 영향을 조사하였다. 또한, hexaconazole 5% SC를 분무한 식물체에서 다양한 시간 간격으로 잎 표본을 채취하여 조사하였다. 분석결과 엽록소, 가용성 단백질, 총페놀함량 등 식물 구성성분과 질산환원효소의 역가에 심한 차이가 있음이 나타났다. 방어관련 효소, 즉 peroxidase의 활성 유도 또한 관찰하였으나, isozyme 양상에는 차이가 없었다. 더욱이, 처리한 식물에서 수확한 토양의 땅콩에서도 탄수화물과 기타 구성성분들의 양적 차이가 보이지 않았다.

색인어 땅콩, 헥사코나졸, 생화학성분, peroxidase