INTEGRATION OF SOME BIOPESTICIDES AND *TRICHOGRAMMA CHILONIS* FOR THE SUSTAINABLE MANAGEMENT OF RICE LEAF FOLDER, *CNAPHALOCROCIS MEDINALIS* (GUENEE) (LEPIDOPTERA: PYRALIDAE)

Muhammad Sagheer[1], Muhammad Ashfaq[1], Mansoor-ul-Hasan[1] and Shahnaz Akhtar Rana[2]
[1]Department of Agri. Entomology, University of Agriculture, Faisalabad
[2]Department of Zoology and Fisheries, University of Agriculture, Faisalabad

Present project was carried out to check the efficacy of microbial and botanical insecticide against *Cnaphalocrocis medinalis,* to assess the effective dose of *Trichogramma chilonis* for a successful control of the rice leaf folder and study the synergistic effect of *Trichograma sp.* and biopesticides for the sustainable control of *C. medinalis*. It is evident from the results that minimum post treatment increase in infestation (2.67%) was recorded against T_3 (1,00,000 eggs) and was, statistically at par, with that recorded from T_4 (1,25,000 eggs) with an increase in percent infestation of 3.683%. These differed significantly from those recorded from T_1 and T_2 with infestation increase of 5.17 and 4.59% respectively. Maximum increase in post treatment infestation was recorded in control treatment. The results of the experiment related to biopesticides shows that neem and Bt gave good results and shown minimum post treatment increase in infestation. 6.087 and 4.093% respectively. The results of the experiment regarding the integration of the best treatments of the *Trichogramma* egg releases and bio-pesticides shows that minimum increase in post treatment infestation (1.373%) was recorded from those plots which were treated with *Trichogramma* + Bt (*Bacillus thuringiensis*) + Neem (T_6) and was statistically at par with T_5 , T_4 and T_2 which showed 2.15, 2.40 and 2.506% increase in post treatment infestation of *C. medinalis*. The control treatment showed maximum increase in post treatment increase of infestation of *C. medinalis* (8.053%).

Keywords: Rice leaf folder, *Cnaphalocrocis medinalis, Trichogramma chilonis,* biopesticides, neem, *Bacillus thuringiensis*

INTRODUCTION

Rice (*Oryza sativa* L.) is a major export commodity and occupies a significant positions in the predominantly agricultural economy of Pakistan. One of the major yields limiting factors is the attack of insect pests that cause 20-30% losses every year (Inayatullah *et al.,* 1986; Salim *et al.,* 2001). The rice leaf folder (RLF), *Cnaphalocrocis medinalis* (Guenée), is a major insect pest of rice. In Pakistan, this pest multiplies enormously, with a severe incidence observed during August-September 1989-90 cropping season. The overall leaf infestation by RLF was seen to be 25 % which reduced the grain yield upto 30% in major rice growing tracts of Punjab. However, at some locations the infestation was found to be 50% (Salim *et al.,* 1991). Changes in the physical environments, cultural practices, multiple cropping patterns, reduced genetic variability of high yielding rice varieties, application of high levels of nitrogenous fertilizer and a prophylactic use of pesticides are the major reasons of the RLF problem (Khan *et al.,* 1989, Dale, 1994. Shepard *et al.,* 1991 and De Kraker *et al*., 2000). Management of RLF using synthetic chemicals has failed because of the pest resistance against them as well as the pest resurgence and environment pollution (Dale, 1994).

Trichogramma chilonis is an effective egg-parasitoid of rice leaf folder. It parasitizes the eggs of rice stem borers also. After rearing it in the laboratory, its population can be augmented in the field to reduce the reliance on the chemicals for the control of lepidopteran pest insect of rice. Khan *et al.,* 2005, reported that the increase in paddy yield after augmentative releases of *T. chilonis*, over control, ranged from 33 to 89.5 kg/acre.

Since the insecticide resistance has limited the effectiveness of many chemical insecticides, an intensive effort has been made to find out alternate methods of control (Senthil Nathan *et al*., 2004). Botanical insecticides and microbial pesticides are highly effective, safe, and ecologically acceptable. Plants produce a diversity of biologically active substances that affect the growth and development of other organisms and can provide protection against the herbivores. These plant products discourage or prevent an attack from the non-adapted organisms and play an important role in the ecology and physiology of phytophagous insects (Sukumar, 1993). *Bacillus thuringiensis* (*Bt*) Berliner is a naturally occurring gram positive, spore forming soil bacterium, and preparations containing *Bt* are widely used as microbial pesticides in agriculture and forestry (Gill *et al*., 1992).

Chemical preparations from the leaves and seeds of the Indian neem tree, *Azadirachta indica* A. Juss. (Meliaceae), have been shown to have deleterious effects on the insects (Schmutterer, 1990). Neem seed kernel extracts (NSKEs) have suppressed the feeding, growth, and reproduction aspects of the pest insects (Ascher *et al*., 1984) and have, thus, been used in many integrated pest management (IPM) programs It is generally believed that the bioactivity of nm is due to its complex limonoids (Mordue and Blackewell, 1993; Mordue and Nisbet, 2000; Huang *et al.,* 2004), but the spectrum and level of its efficacy are yet unknown against the rice leaf folder in the field. Similarly fruit extract of draik, *Melia azedarach*, elicit a variety of effects, such as, antifeedant, growth retardation, reduced fecundity, moulting disorders, morphogenetic defects, and changes of behaviour (Schmidt *et al,.* 1997 and Schmidt *et al.,* 1998).

Present project was carried out to check the efficacy of microbial and botanical insecticide against *Cnaphalocrocis medinalis,* to assess the effective dose of *T. chilonis* for a successful control of the rice leaf folder and study the synergistic effect of *Trichogram*a *sp.* and biopesticides for the sustainable control of *Cnaphalocrocis medinalis*

MATERIALS AND METHODS

Experiments were conducted on the Experimental farms of University of Agriculture, Faisalabad. The nursery of super basmati was sown on June 05, 2006 on well prepared raised beds and about one month old seedlings were transplanted in Randomized Complete Block Design with three replications, in the fields with normal spacing of 9 inches @ two seedlings per hill. All the recommended agronomic practices were adopted during the experimentation.

Use of *Trichogramma sp.*

Trichogramma sp. reared on the eggs of *Sitotroga cerealella* and parasitized, egg-pasted cards were applied under different treatments (75000, 100000, 125000, 150000 eggs) to check their efficacy against RLF. Data were collected again on the basis of damaged/folded leaves.

Use of Biopesticides

Extracts from the leaves and seeds of *Azadirachta indica* and *Melia azadirach* (Darek) were obtained by soaking them in boiled water for two hours. This soak, was left for two days and the extract sieved through muslin cloth. After the calibration of area for each treatment, the extract were sprayed over to the cultivar, sown in RCBD. The experiment was repeated thrice.

Formulations of *Bacillus thuriengiensis,* and *Sacchalaropolyspora spinosa* (spinosad) were also used as microbial insecticide. Recommended dose of the Bt and spinosad were used in triply replicated experiment. Data were collected, one week after application of bio-pesticides, on the basis of damaged or folded leave by using following formula.

$$\text{Folded leaves }\{\%\} = \frac{\text{No. of infested leaves per hill}}{\text{No. of total leaves per hill}} \times \frac{\text{No. of infested hills}}{\text{No. of hills in sample area}} \times 100$$

Integration of *Trichogramma* and Biopesticides

Best treatments of egg release of *Trichogramma,* botanical and microbial insecticides were selected and next year (2007) were used alone and in integration with each other to control the rice leaf folder in a sustainable manner. For integration studies following treatments were used

T1= *Trichogramma* (1,00,000 eggs/acre)
T2= Neem
T3= Bt (*Bacillus thuringiensis*)
T4= *Trichogramma* +Bt
T5= *Trichogramma* + Neem
T6= Neem + Bt + *Trichogramma*
T7= Control

RESULTS

Table1 shows the efficacy of different doses of egg releases of *Trichogramma sp.* against *Cnaphalocrocis medinalis*, this table reveals a significant difference among the treatments. The means were compared by Tukey HSD (P=0.05) given in Table-2. It is evident from the results that minimum post treatment increase in infestation (2.67%) was recorded against T_3 (1,00,000 eggs) and was, statistically at par, with that recorded from T_4 (1,25,000 egg) with an increase in percent infestation of 3.683%. These differed significantly from those recorded from T_1 and T_2 with infestation increase of 5.17 and 4.59% respectively. Maximum increase in post treatment infestation was recorded in control treatment.

Table 1. Analysis of variance for the data regarding the post treatment increase in infestation caused by *Cnaphalocrocis medinalis* (Guenee) against different bio-insecticides in rice crop

SOV	Degree of Freedom	SS	MS	
Replications	2	1.0301	0.5151	3.512 $^{N.S.}$
Treatments	4	67.6449	16.9112	115.322 **
Error	8	1.1731	0.1466	
Total	14	69.8482		

Table 2. Means of the data regarding post treatment increase in infestation by rice leaf folder against different biopesticides

Treatments	Means of percent increase in infestations of RLF ± Std. Error
Bacillus thuringensis	4.093 ± 0.175 a
Spinosad	7.833 ± 0.139 b
Neem	6.087 ± 0.534 ab
Darek	8.850 ± 0.150 bc
Control	10.157± 0.094 c

Table-3 indicates the efficacy of different bio-pesticides (botanical + microbial) against *Cnaphalocrocis medinalis*, this table also reveals significant difference among the treatments. The means were compared by Tukey HSD (P=0.05) given in Table-4. It is evident from the results that neem and Bt showed good results and shown minimum post treatment increase in infestation. 6.087 and 4.093% respectively. These differed significantly from those recorded from T2 and T4 with infestation increase of 7.833 and 8.850 % respectively. Maximum increase in post treatment increase in infestation (10.157%) was recorded in control treatment.

Table 3. Analysis of variance for the data regarding the post treatment increase in infestation caused by *Cnaphalocrocis medinalis* (Guenee) against egg releases of *Trichogramma chilonis* in rice crop.

SOV	Degree of Freedom	SS	MS	F. Ratio
Replications	2	0.3242	0.1621	0.418 N.S.
Treatments	4	66.6916	16.6729	42.9882 **
Error	8	3.1028	0.3878	
Total	14	70.1186		

Table 4. Means of the data regarding post treatment increase in infestation by rice leaf folder against different egg releases of *Trichogramma chilonis* in rice crop

Trichogramma Egg releases	Means of percent increase in infestations of RLF ± Std. Error
50,000	5.170 ± 0.276 b
75,000	4.59 ± 0.326 b
1,00,000	2.670 ± 0.340 a
1,25,000	3.683 ± 0.355 ab
Control	8.856 ± 0.383 c

The results of the experiment regarding the integration of the best treatments of the *Trichogramma* egg releases and bio-pesticides are given in Table-5 which shows that there is significant difference among treatments of this experiment. The means compared by Tukey HSD (P=0.05) are given in Table-6. The results shows that minimum increase in post treatment infestation (1.373%) was recorded from those plots which were treated with *Trichogramma* + Bt + Neem (T_6) and was statistically at par with T_5 , T_4 and T_2 which showed 2.15, 2.40 and 2.506% increase in post treatment infestation of *C. medinalis*. The control treatment showed maximum increase in post treatment increase of infestation of RLF (8.053%).

Table 5. Analysis of variance for the data regarding the post treatment increase in infestation caused by *Cnaphalocrocis medinalis* (Guenee) against different treatments of integration of *Trichogramma* and biopesticides in rice crop

SOV	Degree of Freedom	SS	MS	F. Ratio
Replications	2	0.9506	0.4753	1.2487
Treatments	6	103.1666	17.1944	45.1725
Error	12	4.5677	0.3806	
Total	20			

Table 6. Means of the data regarding post treatment increase in infestation by rice leaf folder against different treatments of integration of *Trichogramma* and biopesticides in rice crop

Treatments	Means of percent increase in infestations of RLF
Trichogramma	3.900000 ±0.258650
Bt	2.506667 ±0.299462
Neem	5.816667±0.390142
Trichogramma + Bt	2.400000± 0.252389
Trichogramma + Neem	2.150000±0.506590
Trichogramma +Bt +neem	1.373333±0.317087
Control	8.053333±0.435941

DISCUSSION

The application of biological control is increasing because of greater environment awareness and food safety concerns and due to the failure of conventional chemicals due to development of resistance in insect pests (Dent, 1993). In the present studies integration of bio-pesticides (microbial + Botanical) and egg parasitoid, *Trichogramma sp.* was made for the sustainable management of Rice Leaf folder. Our studies reveals that use of bio-pesticides has enhanced the effectiveness of *Trichogramma chilonis.* Hassan (1994) used *Trichogramma* to control some 28 different caterpillar pest attacking different crops including rice. Mohanraj *et al.,* (1995) used *Tichogramma* for the control of stem borers. In Assam, Punjab, Gujrat and Tamil Nadu, integrated use of biocontrol agents and Bt was effective in reducing rice stem borer (*Scirpophaga incertulas*) populations. Singh *et al.* (2001) reported that BIMP modules at different crop stages were useful in management of rice stem

borers and Rice Leaf Folder. Our results are also in accordance with the results of Khan *et al.,* 2005 who studied the bio-efficacy of *Trichogramma spp.* against leaf folder in rice ecosystem. They reported that all the doses used in the inundative releases of egg parasitoids were found effective over the control but the releases at the rate of 1,00,000 ha^{-1} was found to be superior over the lower dose. Bentur *et al.,* (1994) reported that leaf damage caused by leaf folder has negative correlation with the number of parasitoid releases.

The biopesticides based on fungus *Metarhizium anisopliae* and bacteria *B. thuringiensis* (Bt) were used against stem borer and leaf folder of rice, which have reduced the population of these pests in the laboratory and in the field (Shahid *et al.* 2003). They recommended the use of biopesticides for the control of rice pests with no harmful effect on its predators as in case with chemical pesticides.

Neem seed kernel extract is an antifeedant and insect growth regulator against many insect pests (Schmutterer, 1990; Mordue and Blackwell, 1993). The growth regulatory effect is the most important physiological effect of neem on insects. It is because of this property that neem has emerged as a source of insecticides. Antifeedant activity and inhibition of hormone and enzyme activity have been attributed to the tetranortriterpenoid, azadirachtin, in the extract (Ma *et al.,* 2000; Zabel *et al.*, 2002; thacker *et al.,* 2003, Nathen *et al,* 2004; Nathen *et al.,* 2005a,b). Our results are contradicted with the results of Keshaw, 2000 who reported that neemactin (Azadirachtin 1500 ppm) alone failed to provided adequate protection from rice leaf folder, it was highly effective against brown plant hoppers.

Fruit extracts of *Melia azedarach* elicit a variety of effects in insects, such as growth retardation, reduced fecundity, moulting disorders, morphogenetic defects, and changes in behavior (Ascher *et al.,* 1995). The antifeedant and insecticidal effects of *M. azedarach* extracts are known for many insects (Sexena *et al.,* 1984; Schmidt *et al.,* 1998; Juan *et al.,* 2000).

The conclusion of this study indicate that bio-insecticides (neem and Bt) affect the pest insect and they can used in integration with egg parasitoid, *Trichogramma* to enhance its bio-efficacy against *C. medinalis*. Plant extracts and microbial formulations may be an effective alternative to conventional synthetic insecticides.The use of these biopesticides may play a more prominent role in integrated pest control programmes in future.

REFERENCES

Ascher, K.R.S., M. Eliahu, N.E. Nemny and J. Meisner. 1984. Neem seed kernel extracts an inhibitor of growth and fecundity in *Spodoptera littoralis*. In: H. Schmutterer and K.R.S. Ascher (Editors), *Natural Pesticides from the Neem Tree and Other Tropical Plants. Proceedings of the Third International Neem Conference, Nairobi, Kenya*, Rottach-Egern, Eschborn (1984), pp. 331–344.

Ascher, K.R.S., H. Schmutterer, C.P.W. Zebitz and S.N.H. Naqvi. 1995. The Persian lilac or Chinaberry tree: Source of unique natural products for integrated pest management, medicine, industry and other purposes. VCH, Weinheim, Germany, pp. 605-642.

Bentur, J.S., M.B. Kalode, B. Rajendran and V.S. Patel. 1994. Field evaluation of the egg parasitoid, *Trichogramma japonicum* Ash. (Hymenoptera: Trichogrammatidae) against the rice leaf folder, *Cnaphalocrocis medinalis* (Guen.) (Lepidoptera: Pyralidae) in India. *Z. Angew. Entomol.* 117(3): 257-261.

Dale, D. 1994. Insect Pests of rice plant, their biology and ecology. In: Heinrches, E.A. (Ed.), *Biology and Management of Rice Insects*. Wiley Eastern Ltd., New York: pp 363-485.

De Kraker, R. Rabbinge, A. van Huis, J.C. van Lenteren and K.L. Heong. 2000. Impact of nitrogenous-fertilization on the population dynamics and natural control of rice leaf folders (Lepidoptera: Pyralidae), *Inter. Pest Manag.* 46: 225–235.

Dent, D.R. 1993. The of *Bacillus thuringiensis* as an insecticide. In: Exploitation of Microorganisms (Ed. Gareth jones), Chapman and Hall, London, pp:19-44.

Dhaliwal, G.S., H.N. Shaki, P.S. Gill and M.S. Maskina. 1979. Field reaction of rice varieties to leaf folder at various nitrogen levels. *Intl. Rice Res. Nwwsl.* 4(3): 7.

Garg, D.K., P. Kumar, R.N. Singh and M. Pathak. 2002. Role of parasitoid *Trichogramma japonicum* and other natural enemies in the management of yellow stem borer and leaf folder in basmati rice. *Indian J. Entomol.,* 64(2):117-123.

Gill, E.A. Cowles and P.V. Pietrantonio. 1992. The mode of action of *Bacillus thuringiensis* endotoxins, *Annu. Rev. Entomol.* (37): 615–636.

Hassan, S.A.1994. Strategies to select *Trichogramma* species for use in biological control. *In: Biological control with egg parasitoids,* (Eds.): E. Wajnberg and S.A. Hassan, pp. 55-71. Oxon, U.K., CAB International.

Huang Z., P. Shi, J. Dai and J. Du. 2004. Protein metabolism in *Spodoptera litura* (F.) is influenced by the botanical insecticide azadirachtin, *Pest. Biochem. Physiol.,* 80: 85-93.

Inayatullah, C., A. Rehman and M. Ashraf. 1986. Management of insect pests of paddy in Pakistan. Progressive Farming. 6(1): 54-62.

Islam, Z. and A.N.M.R. Karim. 1997. Leaf folding behaviour of *Cnaphalocrocis medinalis* (Guenee) and *Marasmia patnalis* Bradley and the influence of rice leaf morphology on damage incidence. Crop Protection 16(2): 215-220.

Juan, A., A. Sans and M. Rabia. 2000. Antifeedant activity of fruit and seed extracts of *Melia azedarach* and *Azadirachta indica* on larvae of *Sesamia nonagrioides*. *Phytoparasitica* 28:311-319.

Kashaw, R.S. 2000. Efficacy of insecticides and their combination with Neemactin (Azadirachtin 1500 ppm) against major pests of rice in Konkan region. *Pestology*, 24(5): 62-63.

Khan, Z.R., B.P. Rueda and P. Caballero. 1989. Behavioral and physiological responses of rice leaf folder, *Cnaphalocrocis medinalis to selected wild rices, Entomol. Exp. Apli,* 52:7-13.

Khan, M.A. and S. Kumar. 2005. Bio-efficacy of *Trichogramma* spp. Against yellow stem borer and leaf folder in rice ecosystem. *Ann. Plant Prot. Sci.* 13(1):97-99.

Ma, D.L., G. Gordh and M.P. Zalucki. 2000. Survival and development of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) on neem (*Azadirachta indica* A.Juss.) leave. Aus. J. Entomol. 39:208-211.

Mishra, B.K., P.R. Mishra and S.M.A. Mandal. 2002. Screening of rice cultivars for resistance to leaf folder. *Indian J. Entomol*., 64(1):68-72.

Mohanraj, P., K. Veenakumari and A.B. Mandal. 1995. Biocontrol of yellow stem borer using *Trichogramma*–a parasitoid native to Andamans. *Rice Biotechnology,* Quarterly, USA, 23: 9-10.

Mordue, A.J. and A. Blackwell. 1993. Azadirachtin: an update, *J. Insect Physiology*., 39:903-924.

Mordue, A.J. and A. J. Nisbet. 2000. Azadirachtin from the neem tree *Azadirachata indica* its action against insects. *An. Sco. Entomol. Brasil*, 29: 615-632.

Nathan, S.S., P.G. Chung and K. Murugan. 2004. Effect of botanicals and bacterial toxin on the gut enzyme of *Cnaphalocrocis medinalis*, *Phytoparasitica,* 32: 433–443.

Nathan, S.S. 2005. Effects of *Melia azedarach* on nutritional physiology and enzyme activities of the rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae). *Pestic. Biochem. Physiol*., 84: 98-108.

Nathan, S.S., K. Kalaivani, K. Murugan and P.G. Chung. 2005a. The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenee) the rice leaffolder. *Pestic. Biochem*. *Physiol.* 81:113-122.

Nathan, S.S., K. Kalaivani, K. Murugan and P.G. Chung. 2005b. Efficacy of neem limonoids on *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae) the rice leaf folder. Crop Prot. 24: 760-763.

Ramazan, M., M. Salim, A. Rehman, S. Hussain and M. Akhtar. 2005. Effect of rope dragging on incidence of rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae). *Pest. Biochem. Physiol*., 84: 98-108.

Rehman, A., M. Saleem, M. Ramzan and M. Akram. 2005. Some bio-ecological studies on leaf folder: A major pest of rice in Pakistan. *Proceedings of the International Seminar on rice crop.* October, 2-3, 2005, pp.262-274.

Salim, M., A. Rehman and M. Ramzan. 1991. Leaf folder outbreaks in Punjab, Pakistan. *IRRN*. 16(1): 22.

Salim, M., S.A. Masud and M. Ramzan. 2001. Integrated pest management of Basmati rice. *Rices of the world: Breeding, Production and Marketing*, FAO, Rome, Italy.

Saxena, R.C., P.B. Epino, W.T. Cheng and B.C. Puma. 1984. Neem, chinaberry and custard apple: antifeedant and insecticidal effects of seed oils on leafhopper and planthopper pests of rice. In: H. Schmutterer, K.R.S. Ascher (Eds.), Proceedings of Second International Neem Conference. Rauischholzhausen, Germany, pp. 403-312.

Schmidt, G.H., A.A.I. Ahmed and M. Breurer. 1997. Effect of *Melia azedarach* extract on larval development and reproduction parameters of *Spodoptera littoralis* (boisd.) and *Agrotis ipsilon* (Hufn.) (Lepidoptera: Nuctuidae) *Anz. Schadlingskde., PflSchutz, Umweltschutz*,70:4-12.

Schmidt, G.H., H. Rembold, A.A.I. Ahmed and M. Breuer. 1998. Effect of *Melia azedarach* fruit extract on juvenile hormone titer and protein content in the haemolymph of two species of noctuid lepidopteran larvae (Insecta: Lepidoptera: Noctuidae). *Phytoparasitica*, 26: 283-292.

Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*, *Annu. Rev. Entomol.,* 35: 271–297.

Shahid A.A., I.A. Nasir, A.U. Zafar, A. Sumrin, B. Chaudhry and S. Riazuddin. 2003. The Use of CAMB Biopesticides to control Pests of Rice (*Oryza sativa*). Asian J. Plant Sci., 2(15-16): 1079-1082.

Shepard, B.M., Z.R. Khan, M.D. Pathak and E.A. Heinrichs. 1991. Management of insect pests of rice in Asia. *In:* D. Pimentel, Editor, *Handbook of Pest Management in Agriculture* (second ed.), CRC Press, pp. 225–278.

Singh S.P., N.S. Rao, S. Ramani and J. Poorani. (Eds). 2001. Annual Report 2000-01, Project Directorate of Biological Control, Bangalore, Bangalore, India; PDBC, 218 pp.

Sukumar, K. 1993. Role of allelochemicals in the control of phytophagous insects. *In*: T.N. Ananthakrishnan and A. Raman, (Editors), *Chemical Ecology of Phytophagous Insects*, Oxford and IBH, New Delhi, pp. 82–89.

Thacker, J.R.M., W.J. Bryan, C. McGinley, S. Heritage and R.H.C Strang. 2003. Field and laboratory studies on the effects of neem (*Azadirachta indica)* oil on the feeding activity of the large pine weevil *(Hylobius abietis* L.) and implications for pest control in commercial conifer plantations. Crop Prot. 22: 753-760.

Thamrin, M. and H. Rosmini. 1993. Rice resistance to leaf folder in tidal wet lands. Intl. Rice Res. Notes, 18(1): 27 (R.A.E./A.82 (6): 5342,1994).

Zabel, A., B. Manojlovic, S. Rajkovic, S. Stankovic and M. Kostic. 2002. Effect of neem extract on *Lymantria dispar* L. (Lepidoptera: Lymantriidae) and *Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae). J. Pest. Sci. 75: 19-25.