Lethal and sublethal effects of spirotetramet and flubendiamide against leaf worm, S*podoptera litura* under laboratory conditions (#81037)

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Lethal and sublethal effects of spirotetramet and flubendiamide against leaf worm, *Spodoptera litura* under laboratory conditions

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Leaf worm, *Spodoptera litura* (Lepidoptera: Noctuidae) is a the notorious pest of many economically important cultivated crops and vegetables causing severe economic lossesdamages from 50-100%. In most of the crops, damage arises due to voracious feeding by the larvae and leads to the skeletonizing of leaves. Toxicological studies were performed to estimate Lethal and sublethal levels toxicity of flubendiamide and spirotetramet were observed against the second instar larvae of *Spodoptera litura* under laboratory conditions. Toxicological studies were performed for the estimation of lethal and sublethal levels for these two insecticides for further studies. Effects of these estimated values were assessed on different biological traits of S.litura including duration of life stages, survival, reproductive potential and progeny success.
These estimated values were used to expose the second instar larvae at different lethal and sublethal levels to observe their effects on different biological traits including life duration, survival and next generation potential. Both flubendiamide and spirotetramet showed toxic response responses against the second instar larvae of *S. litura* under laboratory conditions. Lethal and sublethal levels of these tested insecticides showed drastic changes in larval duration and survival rate. Exposure to test insecticides resulted in negative effects on the demography of s. litura as longer life cycle and decreased fecundity. Increased larval and adult duration after exposure to these insecticides showed their long term effects on their demographic parameters.
Changes in net reproductive rate and intrinsic rate of increase also helped to decide about the fate of these insecticides. Low reproductiveegg potential and very low hatching percentage due to exposure to test insecticide can were promising to help to manage in

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the next generation of target pest. These two new chemistry insecticides can be recommended for their effective and long termlong-term utilization against this important leaf feeder which may help its management and decrease in economic losses faced by the growers. Their impact in on larval duration and low survival rate at lethal levels guides about their potential of new arsenal in pest control.

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ABSTRACT

Leaf worm, *Spodoptera litura* (Lepidoptera: Noctuidae) is the notorious pest of many economically important cultivated crops and vegetables causing severe economic damages from 50-100%. In most of the crops, damage arises due to voracious feeding by the larvae and leads to the skeletonizing of leaves. Lethal and sublethal toxicity of flubendiamide and spirotetramet were observed against the second instar larvae of *Spodoptera litura* under laboratory conditions. Toxicological studies were performed for the estimation of lethal and sublethal levels for these two insecticides for further studies. These estimated values were used to expose the second instar larvae at different lethal and sublethal levels to observe their effects on different biological traits including life duration, survival and next generation potential. Both flubendiamide and spirotetramet showed toxic response against the second instar larvae of *S. litura* under laboratory conditions. Lethal and sublethal levels of these tested insecticides showed drastic changes in larval duration and survival rate. Increased larval and adult duration after exposure to these

insecticides showed their long term effects on their demographic parameters. Changes in net reproductive rate and intrinsic rate of increase also helped to decide about the fate of these insecticides. Low egg potential and very low hatching percentage were promising to help manage in the next generation. These two new chemistry insecticides can be recommended for their effective and long term utilization against this important leaf feeder which may help its management and decrease in economic losses faced by the growers. Their impact in larval duration and low survival rate at lethal levels guides about their potential of new arsenal in pest control.

Keywords: *Spodoptera litura*; spirotetramet; flubendiamide; sublethal

INTRODUCTION

Leaf worm (*Spodoptera litura* L.) is notoriously polyphagous insect pest widely distributed in South Asia with wide host range of more than hundred host plants (Ahmad *et al*., 2013; Sang *et al*., 2015). During the survey of three different sites in the cotton belt of Southern Punjab, 27 host plant species of S.litura were reported ~~-~~belonging to 25 genera and 14 families including cultivated crops, ornamental, fruits, vegetables and weeds ~~were observed~~ (Ahmad *et al*., 2013). Female lays round or spherical eggs, covered with hairy scales. Larvae also vary in colors and length of full grown larvae is almost 40-45mm having longitudinal bandswith ~~and~~ two dark spots ~~are~~ present on its dorsal side. Adult moth is grayish brown in color (Simmons *et al*., 2018) and egg hatches within 3-5 days and life cycle complete ~~is completed~~ in 5 weeks.

Due to its gregarious feeding behavior, if not managed timely, serious crop damage may occur with reduced crop yield (Dhir *et al.*, 1992; Ahmad *et al.*, 2009). It causes considerable losses during the reproductive stages of the crop (Singh and Sachan, 1992). Depending upon feeding on different host plants, it has gained different names like tobacco cutworm, tobacco caterpillar, Indian leaf worm and cluster caterpillar (Ahmad *et al.*, 2007a). In Pakistan, its infestation starts at the end of March and sustain up to November (Sayyed *et al.*, 2008). In the cotton growing areas, it is abundantly found in September and October (Islam *et al.*, 1984).

Among lepidopteran insect pests, *S. litura* was the first pest that developed resistance (Srivistava and Joshi, 1965) against organophosphates (Vijayavaghavan and Chirta, 2002) and pyrethroids (Babu and Santharam, 2002; Sudhakar and Dhingra, 2002). Extensive use of chemicals resulted in the failure of control, pest resurgence and many health hazards (Ahmad *et al*., 2007; Khan and Mehmood, 1999. Insecticide resistance to almost all the available insecticides ~~has~~ have been previously recorded based on laboratory and field studies (Kranthi *et al*., 2002). Long field exposure to different insecticides resulted in the development of resistance (Ahmad *et al.*, 2009). Use of organophosphates, pyrethroids and carbamates for more than two decades created the best environment for resistance development against these conventional insecticides and resulted in failure of effective control (Ramakrishnan *et al.*, 1983; Wu *et al*., 1995; Ahmad *et al.*, 2009). However, the mixture of insecticides (chlorpyrifos, profenofos and fipronil) was found to be an effective ~~alternate~~ alternative against *S. litura* management (Ahmad *et al*., 2009). Field control became more difficult and expensive for later larval instars owing to their high pesticides tolerance (Kim *et al.*, 1998).

Chemical control still persisted as the common method because of its ease of application and quick pest control (Peter and David 1988; Kumar and Parmar, 1996). Although, insecticides give rapid control yet there are certain disadvantages like disruption of natural balance and health hazards. Furthermore, inappropriate application of insecticides at high dose ~~rate~~ rates also leads to the development of resistance and environmental pollution. On the other hand, the sublethal effects of different insecticides influence the biological parameters affecting the larval and pupal duration, mating, pupal weight, fecundity and fertility of eggs. However, adult longevity and pupal weight were not affected by insecticides' application but negatively affected the copulation period (Jasoja, 2002). Fluvalinate and cyhalothrin affected the biological parameters of lepidopteran pests with increased sensitivity of adult male moth in comparison with female moth and changes in the longevity of larval and pupal stages (Abro *et al.*, 1997).

Chemicals used for the control of lepidopteran species have some demographical effects on insect population. Chlorantraniliprole showed reduction in survival of the offspring, fecundity and egg hatching whereas the period of oviposition increased in *Plutella xylostella* with delayed development (Han *et al.*, 2012). Chlorfluazuron when applied on *S. litura* at sublethal rates affected the instar development, pupal moulting and emergence of adult; however, their hazards were higher at lethal dose rates. Similarly, the body weight of larvae and pupae, fertility of female by 49-58% and hatchability reduced by 22-26%. Male fertility was reduced by 65-81% and hatchability by 44-66% with enhanced male sensitivity (Parveen, 2000). Flubendiamide did not exhibit the cross resistance and phytotoxicity at their recommended field doses for *P. xylostella*, *S. litura* and *Pieris sp.* Foliar application of flubendiamide has previously being proposed for the control of lepidopteran pests on vegetables (Khan *et al.*, 2011). Keeping in view the~~of~~ important of role of new chemistry insecticides like flubendiamide and spirotetramet, lethal and sublethal effects on important biological parameters of *S. litura*, were planned to observe the toxicity and to check the lethal and sublethal effects of flubendiamide and spirotetramet on early larval stage at the 2nd instar of *S. litura* against different life history parameters like net fecundity rate, generation time, survival rate etc.

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MATERIALS AND METHODS

Collection and Rearing of *Spodoptera litura*

S. litura larvae were collected ~~Collection of *S. litura* (about 200, 3rd to 4th instar) larvae was made~~ from cauliflower field crop of Rawalpindi by hand picking from random population collection method. These larvae were kept in a plastic jar lined with cauliflower leaves as food during transportation to the laboratory. Larvae were reared in plastic jars on~~with~~ castor leaves provided daily after~~on daily basis after~~ cleaning the previous semi-consumed leaves and frass. The larvae stopped feeding a day before pupation and let undisturbed to pupate. Pupae were collected two days after pupation or when their cuticle got matured and then placed ~~them~~ in another plastic box ~~which~~ lined with tissue paper ~~in order to~~to avoid any damage or moisture problem. Newly emerged moths were kept in separate plastic jars and nappy liner strips were ~~hanged~~ hung as a substrate for laying eggs. For adults, 10% honey solution was provided and changed as per need. Egg batches were collected daily and kept in separate plastic Petri-dish ~~labeled~~ labelled accordingly. Egg batches near to hatch were placed in sandwich of castor leaves for their easy and direct access to food for maximum survival in early instar and decrease the mortality chances. These larvae were reared till their moulting to the second instar desired for the experiment initiation.

Insecticides

Commercial formulations of insecticides namely flubendiamide (Belt® 48 SC, Bayer Crop Science) and spirotetramat (Movento® 240 SC, Bayer Crop Science) were kindly provided by the Bayer Crop Science, Pakistan to observe their possible impact on different biological traits of leaf worm.

Bioassays

Acute Toxicity Studies

Leaf dip bioassay with no choice was used in order to estimate initial toxicity against field population of *S. litura*. Stock solution of insecticides was prepared based on their field dose rates. From the stock solution, 5-6 serial ~~concentration~~ concentrations with half dilution factor were prepared and considered as treatments. Leaves of castor plant were washed, dried, and cut into 5 cm diameter discs and dipped in prepared concentration for 10 to 15 seconds. After drying the leaves with insecticide solution in fume hood, five larvae per Petri dish lined with moist filter paper were released. Forty early second instar larvae per treatment were selected and mortality was recorded as end point with 24 hours ~~interval~~ intervals till the fifth day. Same number of larvae was released on water treated leaf discs as control.

Chronic Toxicity Studies

For demographic studies, acute toxicity data of 72 hours was used to analyze the values of LC_{10}, LC_{25}, LC_{50} and LC_{75} for both the insecticides. Forty 2nd instar larvae were exposed at each concentration level with same numbers in untreated control. Each insect was treated as a replicate and data were observed on daily basis till hatching percentage of eggs from generation obtained. Biological parameters like numbers of larval ~~moult~~moults, larval duration, pupal and adult duration, hatching percentage and mortality at all the levels were observed.

Data Analysis

For second instar larvae mortality on the basis of concentration was assessed by Probit analysis after correcting the observed data with the control mortality following Abbott (1925) and Finney (1971) with the help of statistical package POLO-PC specially used for such toxicological studies LeOra (1987). Percent survival rate of larva, pupa and adult, pupa and adult deformation, reproductive~~egg~~ potential per pair and percentage hatching was observed for estimation of the intrinsic rate of increase (r_m) following Walthall and Stark (1997).

Results

Lethal and sublethal toxicity and the possible effects of flubendiamide and spirotetramet on *S. litura* were observed by leaf dip method under laboratory conditions. Lethal concentrations at 10, 25, 50 and 75 percentage (LC_{10}, LC_{25}, LC_{50} and LC_{75}) kill for both insecticides were estimated for five consecutive days of exposure with mortality as a n endpoint. For flubendiamide, comparative ratio for 10% LC value revealed almost five times increase in toxicity from day one to five. It was 25 and 144 times higher for 25%LC value, 16 and 500 times higher for 50%LC value and 15 and 2480 times higher for 75%LC value, respectively when compared with the least respective LC value for each level. For spirotetramet, comparative ratio for 10% LC value revealed almost four and two times increase in toxicity from day one to five. It was 2 and 8 times higher for 25%LC value, 23 and 228 times higher for 50%LC value and 2 and 35 times higher for 75%LC value, respectively when compared with the least respective LC value for each level. Overall comparison of these two insecticides showed comparatively higher toxicity of flubendiamide than spirotetramet against this leaf feeder (Table 1).

For biological studies, lethal level of LC_{75} was excluded after initial testing during which almost all the exposed insects died. High mortality observed at LC_{75} prompted us ~~This high mortality observed made us~~ to select the sublethal level of LC_{10} to incorporate for the possible impact of another low concentration level after LC_{25} as planned initially. Impact of flubendiamide on three levels of LC_{10}, LC_{25} and LC_{50} in comparison with untreated control showed variable changes in the development of the surviving insects ~~at these three levels.~~ Duration After the release of the same number of second instar larvae of *S. litura* on these four levels, duration of second larval instar get ~~shorten~~ shortened a bit from sublethal (LC_{10}, LC_{25}) to lethal (LC_{50}) concentration as compared to control. In third larval instar, lethal level significantly decreased the duration in comparison to sublethal levels and control which were almost similar

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Impact of spirotetramet on three levels of LC_{10}, LC_{25} and LC_{50} in comparison with untreated control also showed variable changes in development of the surviving insects at these three levels. After the release of same number of second instar larvae of *S. litura* on these four levels, duration of second larval instar get shorten a bit from sublethal (LC_{10}, LC_{25}) to lethal (LC_{50}) concentration as compared to control with almost no change for the third larval stage. For fourth larval instar, shortest time was taken for the lethal concentration which lasted only for one day whereas the sublethal levels showed slightly increased duration when compared to control. All test insects exposed to lethal concentration died during fourth instar(or after moulting to fifth instar plz confirm)with 100 percent mortality. ~~There appeared to be no survival after fourth larval instar and all the exposed insects died at the lethal level~~. The surviving insects for sublethal concentrations showed slightly more time to complete fifth and sixth larval instars, and pre-pupa stage than control, however, time duration was slightly decreased as compared to control during pupal stage ~~it slightly get shorten for pupa but~~ extended for adult stages (Table 3).

Under flubendiamide stress, there appeared to be a variable net reproductive rate under sublethal

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182 and lethal concentration as compared to control. Lethal concentration level was similar to control

population but sublethal levels increased net reproductive rates. There appeared to be very low generation time and increased intrinsic rate of increase at the tested lethal and sublethal concentration levels in comparison to control. Lethal and sublethal stress caused by spirotetramet showed increased net reproductive rate with increased concentration levels and generation time and intrinsic rate of increase in reverse orders (Table 4). Lethal and sublethal stress of both insecticides resulted in very low egg hatching percentage in comparison to control, however, eggs laid at the sublethal concentration levels of spirotetramet only and a fraction at the lower sublethal level of flubendiamide. These reproductive poptential egg potentials and their hatching percentage revealed a plunge drastic decline in the number of offspring population of *S. litura* under lethal and even sublethal concentration levels of both insecticides. Mean relative growth rate for flubendiamide remained very low for lethal and sublethal levels and quite higher at sublethal levels but no survival at lethal level when compared to control (Table 5).

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Commented [MRA9]: Generation time is looking like wrong its lower than 3 days in total which cant be possible please check table 4

Discussion

Insecticides are the killing agent which used to plays drastic effect to kill the pests of different field and vegetable crops. Lepidopteran pests grow rapidly due to short life span and high reproductive potential. These pests cause economical damage to many crops and household things. Different pesticides are used to kill these pests which are an easy, short and cheap way to kill the pest of damaging entities. Long-term use of insecticides provoked resistance in some pests and eventually failure in managing the specific pest in the field. Some pests got resistance for a long time using of different insecticides and not managed by insecticides which having resistance against them. This is common for *S. litura* that use of same insecticides for long duration may result in failure of control the pest.

Shorter life span and high reproductive potential of insects makes them very efficient to increase their number in short time. This high number in population creates problem when they behave like insect pests of economically important crops necessary for our survival and growth. Most commonly used control methods include host plant resistance, biological control and chemicals as pesticides to kill these insect pests. The latter method of pest control is common in Asian countries facing more pest problems due to good climatic conditions and variety of food resources for multiplication of such insect pests. Their wide and long term use has resulted in different problems including insecticide resistance and resurgence. Lethal and sublethal effects of newly introduced insecticides provides more detailed and effective utilization of these chemicals for long term management and weak links to target these insect pests including this important leaf feeder of many economically important crops persisting for a long duration on different crops (Sayyed *et al*., 2008).

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Present studies revealed decreased larval duration and mean relative growth rate for both insecticides with increased concentration levels. Although there was some egg potential at sublethal levels of spirotetramet and flubendiamide yet there remained a very low survival rate for the next generation. Such drastic decrease in number of such insects helps to manage them under less insecticide use and at the desired recommended rate of application. Such changes have previously been observed to minimize the use of pesticides against insect pests and make our food and environment less hazardous (Stark *et al*., 1997). Increase in larval mortalities not only reduces the losses at that particular crop stage when that insecticide applied but also decreased them for the coming generations (Thakur *et al*., 2013).

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Emamectin has proved to be more effective than indoxacarb, lufenuron and spinosad whereas abamectin was the least effective in previous lethal studies for this pest (Ahmad *et al*., 1995; 2005). It has also been observed toxic to *S. litura* in the surrounding country field strains (Karuppaiah and Chitra, 2013). Emamectin was previously found toxic to beneficial insect like

Chrysoperla carnea in local strains of Pakistan; however, flubendiamide was moderately toxic and considered safer (Hussain *et al*, 2012). There is need to know more about the lethal and sublethal response which make present study to compare these new insecticides with novel mode of action. Such studies will be helpful for future application of these insecticides against this important leaf feeder and other economic insect pests.

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Demographic toxicity is becoming a new field of toxicology (Stark and Wennergren, 1995; Forbes and Calow, 1999) because it covers all effects including the lethal and sublethal that an exposed insect might have on its population. The studies usually performed on complete life cycle need to be obtained under pesticide stress (Stark and Banks, 2000, 2003). The demography and other parameters of life for estimation of toxicity should be adopted more widely.

References

Abbott, S. W. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Abro, G. H., J. Memon and T. S. Syed. 1997. Sub-lethal effects of cyhalothrin and fluvalinate on biology of *Spodoptera litura* (F.). Pak. J. Zool., 29(2): 181-184.

Ahmad, M., M. I. Arif and Z. Ahmad. 1995. Monitoring insecticide resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. J. Econ. Entomol., 88: 771–776.

Ahmad, M., M. A. Saleem and M. Ahmad. 2005. Time oriented mortality in leaf worm, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) by some new chemistry insecticides. Pak. Entomol., 27: 67-70.

Ahmad, M. 2007. Potentiation / antagonism of pyrethroids with organophosphate insecticides in *Bemisia tabaci* (Homoptera: Alerodidae). J. Econ. Entomol. 100: 886-893.

Ahmad, M., I. Arif and M. Ahmad. 2007a. Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. Crop Prot., 26(6): 809–817.

Ahmad, M., M. A. Saleem and A. H. Sayyed. 2009. Efficacy of insecticide mixtures against pyrethroid and organophosphate resistant populations of *Spodoptera litura* (Lepedoptera: Noctuidae). Pest Manag. Sci., 65: 266-274.

Ahmad, M., A. Ghaffar and M. Raffiq. 2013. Host plants of leaf worm, *Spodoptera litura* (Fabricius) (Lepidoptera : Noctuidae) in Pakistan. Asian. J. Agric. Biol., 1(1): 23-28.

Byrne, F. J. and N. C. Toscano. 2001. An insensitive acetyl cholinesterase confers resistance to methomyl in the beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae). J. Econ.Entomol., 94: 524–528.

Dhir, B. C., H. K. Mohapatra and B. Senapati. 1992. Assessment of crop loss in groundnut due to tobacco caterpillar, *Spodoptera litura* (F.). Indian J. Plant Prot., 20(7-10): 215- 217.

Finney, D. J. 1971. Probit Analysis, 3rd ed. Cambridge University press, UK.

Han, W., S. Zhang, F. Shen, M. Liu, C. Ren and S. Gao. 2012. Residual toxicity and sublethal effects of chlorantraniliprole on *Plutella xylostella* (Lepidoptera: Plutellidae). Pest Manag. Sci., 68: 1184-1190.

Hussain, D., A. Ali, R. Tariq, M. M. Hassan and M. Saleem. 2012. Comparative toxicity of some new chemistry insecticides on *Chrysoperla carnea* (Stephans) under laboratory conditions. J. Agric. Res. 50(4): 509-515.

Islam, W., K. N. Ahmad and O. I. Joarder. 1984. Timing and extent of damage caused by insect pest of green gram (*Vigna radiate* L.) in Bangladesh. Crop Prot., 3: 343-348.

Jasoja, M. L. 2002. To study the sub-lethal effect of insecticides on *Spodoptera litura* F. under laboratory conditions. M.Sc. thesis. SAUT, Tandojam (Pakistan).

Karuppaiah, V. and S. Chitra. 2013. Relative toxicity of newer insecticide molecules against *Spodoptera litura*. Annals. Plant Prot. Sci. 21 (2): 305-308.

Kim, Y. G., J. R. Cho, J. N. Lee, S. Y. Kang, S. C. Han, K. J. Hong, H. S. Kim, J. K. Yoo and J. O. Lee. 1998. Insecticide resistance in the tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). J. Asia-Pacific Entomol., 1: 115-122.

Khan, A. and R. Mehmood. 1999. Cotton crop survey report, 1998-99; Pakistan Central Cotton Committee, Multan, Pakistan.

Khan, R. R., S. Ahmed and S. Nisar. 2011. Mortality responses of Spodoptera litura (Fab.) (Lepidoptera: Noctuidae) against some conventional and new chemistry insecticides under laboratory conditions. Pak. Entomol., 33(2): 147-150

Kranthi, K. R., D. R. Jadhav, S. Kranthi, R. R. Wanjari, S. S. Ali and D. A. Russell. 2002. Insecticides resistance in five major insect pests of cotton in India. Crop Prot., 21: 449-460.

Kumar, J and B. S. Parmar, 1996. Physicochemical and chemical variation in neem oils and some bioactivity leads against *spodoptera litura* F. J. Agric. Food Chem., 44: 2137-2143.

LeOra. 1987. POLO-PC, A user's Guide to Probit or Logit Analysis. LeOra software, Barkeley, CA, USA. 33 pp.

Parveen, F. 2000. Effects of sublethal dose of chlorfluazuron on testicular development and spermatogenesis in the common cutworm, *Spodoptera litura*. Physiol. Entomol., 25: 315-323.

Peter, C and V. David. 1988. Residual toxicity of some insecticides on groundnut to the first and third instars larvae of *Spodoptera litura* F. (Lepidoptera: Noctuidae*).* Trop. Pest Manag., 34: 24-26.

Ramakrishnan, N., V. S. Saxena and S. Dhingra, 1983. Insecticide resistance in the population of *Spodoptera litura* (Fab.) in Andhra Pradesh. Pestic., 18: 23-27.

Sang, S., Shu, B., Yi, X., Liu, J., Hu, M. and Zhong, G., 2016. Cross0resistance and baseline susceptibility of Spodoptera litura (Fabricius)(Lepidoptera: Noctuidae) to cyantraniliprole in the south of China. *Pest Management Science*, *72*(5), pp.922-928.

Simmons, A.M., Wakil, W., Qayyum, M.A., Ramasamy, S., Kuhar, T.P. and Philips, C.R., 2018. Lepidopterous Pests: Biology, Ecology, and Management. In *Sustainable Management of Arthropod Pests of Tomato* (pp. 131-162). Academic Press.

Singh, K. N. and G. C. Sachan. 1992. Assessment of yield loss due to insect pest at different growth stages of groundnut in Pantnagar, Uttar Pradesh, India. Crop Prot., 11: 414-418.

Srivastava, B. K. and H. C. Joshi. 1965. Occurrence of resistance to BHC in *Prodenia litura* (Fab.) (Lepidoptera: Noctuidae). Indian J. Entomol., 27: 102-104.

Sudhakar, K. and D. Dhingra, 2002. Effect of combination of sublethal concentration of chemical and microbial insecticides to different larval instars of *Spodoptera litura* (Fab.). J. Pest Res., 14: 32-39.

Sayyed, A. H., M. Ahmad and M. A. Saleem. 2008. Cross-resistance and genetics of resistance to indoxacarb in *Spodoptera litura* (Lepidoptera: Noctuidae). J. Econ. Entomol., 101: 472–479.

Stark, J. D, L. Tanigoshi, M. Bounfour and A. Antonelli. 1997. Reproductive potential: its influence on susceptibility of species to pesticides. Entomol. Environ. Safety, 37: 273-279.

Thakur, A., S. Kaur, A. Kaur and V. Singh. 2013. Enhanced resistance to *Spodoptera litura* in endophyte infected cauliflower plants. Environ. Entomol., 42(2): 240-246.

Vijayaraghavan, C. and K. C. Chitra. 2002. Total protein and free aminoacid content of *Spodoptera litura* (Fab.) due to botanicals and conventional insecticides. Ind. J. Econ. Entomol., 64: 92-95.

Walthall, W. K. and J. D. Stark. 1997. Comparison of two population level ectotoxicological endpoints: the intrinsic (r_m) and instantaneous (r_i) rates of increase. Environ. Toxic. Chem., 16: 1068-1073.

Wu, S., Y. Gu and D. Wang. 1995. Resistance of the tobacco army moth (*Prodenia litura*) to insecticides and its control. Acta Agric. Shanghai., 11: 39-43.

Table 1 (on next page)

Lethal and sublethal concentration levels of flubendiamide and Spirotetramet against field population of *Spodoptera litura* when tested under laboratory conditions at second larval instar

Table 1: Lethal and sublethal concentration levels of flubendiamide and Spirotetramet against field population of *Spodoptera litura* when tested under laboratory conditions at second larval instar

Insecticides	Time of Obs.	LC_{10} (95% FL)	LC_{25} (95% FL)	LC_{50} (95% FL)	LC_{75} (95% FL)	LC_{90} (95% FL)	Slope± SE	Chi square	P
Flubendiamide	**24hrs**	0.01 (0.00-0.03)	0.14 (0.07-0.31)	3.00 (1.05-26.59)	62.48 (10.12-3824)	959.49 (73.5-539)	0.51±0.11	0.89	0.83
	48hrs	0.01 (0.00-0.02)	0.03 (0.02-0.05)	0.13 (0.09-0.19)	0.54 (0.35-0.93)	1.90 (1.07-4.32)	1.11±0.12	2.23	0.53
	72hrs	0.01 (0.00-0.02)	0.03 (0.01-0.05)	0.09 (0.05-0.16)	0.34 (0.19-0.83)	1.08 (0.50-4.26)	1.20±0.12	3.14	0.37
	96hrs	0.00 (0.00-0.00)	0.01 (0.00-0.01)	0.02 (0.01-0.023)	0.06 (0.04-0.08)	0.16 (0.11-0.30)	1.34±0.17	2.52	0.47
	120hrs	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.01 (0.00-0.01)	0.02 (0.01-0.03)	0.08 (0.05-0.16)	1.12±0.19	2.36	0.50
Spirotetramet	**24hrs**	4.33 (1.20-9.91)	28.3 (13.0-49.2)	227 (142-378)	1831 (973-4641)	11967 (4709-31706)	0.75±0.09	0.59	0.90
	48hrs	3.06 (0.80-7.27)	20.3 (8.92-36.3)	166 (103-271)	1365 (745-3274)	9062 (3686-3680)	0.74±0.09	0.60	0.87
	72hrs	2.03 (0.03-8.03)	6.45 (0.32-18.6)	23.2 (4.58-57.8)	83.9 (32.9-355)	266 (99.0-3583)	1.21±0.13	8.98	0.03
	96hrs	1.42 (0.01-6.27)	4.69 (0.15-14.6)	17.65 (2.65-44.6)	66.3 (24.4-260)	218 (81.6-2818)	1.17±0.13	8.03	0.05
	120hrs	0.98 (0.01-4.50)	3.36 (0.11=10.7)	13.18 (1.92-32.5)	51.7 (19.2-168)	177 (69.5-1627)	1.14±0.13	6.49	0.10

LC_{10}= Lethal concentration (ppm) at 95% level	LC_{25}= Lethal concentration (ppm) at 95% level
LC_{50} = Lethal concentration (ppm) at 95% level	LC_{75} = Lethal concentration (ppm) at 95% level
FL =Fiducial limits at 95% level	SE = Standard Error

Table 2(on next page)

Comparative ratios of lethal and sublethal concentration levels of flubendiamide and Spirotetramet against field population of *Spodoptera litura* when tested under laboratory conditions

Table 2: Comparative ratios of lethal and sublethal concentration levels of flubendiamide and Spirotetramet against field population of *Spodoptera litura* when tested under laboratory conditions

Insecticides	Time of Obs.	LC_{10}	C	LC_{25}	C	LC_{50}	C	LC_{75}	C
Flubendiamide	**24hrs**	0.009	4.5	0.144	144	3.004	500.6	62.48	2840
	48hrs	0.009	4.5	0.032	32	0.131	21.83	0.535	24.31
	72hrs	0.008	4	0.025	25	0.093	15.5	0.338	15.36
	96hrs	0.002	1	0.006	6	0.018	3	0.056	2.54
	120hrs	0.000		0.001	1	0.006	1	0.022	1
Spirotetramet	**24hrs**	4.33	4.42	28.2	8.42	227	17.2	1831	35.4
	48hrs	3.06	3.12	20.3	6.05	166	12.6	1365	26.4
	72hrs	2.03	2.07	6.45	1.92	23.2	1.76	83.9	1.62
	96hrs	1.42	1.45	4.69	1.39	17.6	1.33	66.3	1.28
	120hrs	0.98	1.00	3.35	1.00	13.1	1.00	51.7	1.00

Table 3(on next page)

Toxicological response of lethal and sublethal concentration levels of flubendiamide and Spirotetramet against ~~field population~~ of *Spodoptera litura* for different life history parameters

Commented [MRA13]: Toxicity response on demography was assessed after rearing in lab until the next generation so may be its not a field population please confirm this g

Table To response of lethal and sublethal concentration levels of flubendiamide and Spirotetramet against field population of *Spodoptera litura* for different life history parameters

Insecticides	Conc.	Larval instars (L)						Other life stages		
		1st L ± SE	**2nd L ± SE**	**rd L ± SE**	**4th L ± SE**	**5th L ± SE**	**6th L ± SE**	**Pre pupae ± SE**	**Pupae ± SE**	**Adults ± SE**
Flubendiamide	LC_{10}	3.00±0.00	2.65±0.15	2.03±0.20	3.83±0.27	5.2±0.17	2.4±0.14	1.00±0.00	11±0.62	6.00±0.22
	LC_{25}	3.00±0.00	2.35±0.11	2.06±0.25	5.67±0.09	4.00±0.00	1.33±0.09	1.00±0.00	10.3±0.24	4.00±0.16
	LC_{50}	3.00±0.00	2.05±0.05	1.28±0.09	6.00±0.00	3.00±0.00	3.00±0.00	1.00±0.00	11.0±0.00	1.00±0.00
	Control	3.00±0.00	2.52±0.11	2.33±0.10	2.84±0.16	4.35±0.14	2.31±0.14	1.14±0.05	9.55±0.53	7.95±0.33
Spirotetramet	LC_{10}	3.00±0.00	2.47±0.10	2.28±0.15	3.19±0.27	4.77±0.22	3.04±0.15	1.23±0.09	9.09±0.52	9.06±0.27
	LC_{25}	3.00±0.00	2.47±0.15	2.44±0.14	3.19±0.25	4.5±0.25	2.58±0.22	1.23±0.06	8.58±0.34	8.18±0.34
	LC_{50}	3.00±0.00	2.12±0.06	2.35±0.11	1.00±0.00					
	Control	3.00±0.00	2.52±0.11	2.33±0.10	2.84±0.16	4.35±0.14	2.31±0.14	1.14±0.05	9.55±0.53	7.95±0.33

Table 4(on next page)

Rate of change in demographic parameters of *Spodoptera litura* under lethal and sublethal concentration levels of flubendiamide and spirotetramet

Table 4: Rate of change in demographic parameters of *Spodoptera litura* under lethal and sublethal concentration levels of flubendiamide and spirotetramet

Insecticides	Concentration	Net rate of reproduction (R_o)	Generation time (T) days	Intrinsic rate of increase (r_m)
Flubendiamide	LC_{10}	23.02	2.38	1.31
	LC_{25}	25.51	2.27	1.42
	LC_{50}	18.82	1.97	1.48
Spirotetramet	LC_{10}	19.2	8.63	0.34
	LC_{25}	23	4.73	0.66
	LC_{50}	26.27	2.57	1.27
	Control	18.97	21.11	0.13

R_o=

r_m= o

Table 5(on next page)

Impact of lethal and sublethal concentration levels of flubendiamide and spirotetramet against egg potential, hatching percentage and mean relative growth rate of *Spodoptera litura* field population

Table 5: Impact of lethal and sublethal concentration levels of flubendiamide and spirotetramet against egg potential, hatching percentage and mean relative growth rate of *Spodoptera litura* field population

Insecticides	Concentration	Egg potentials ±SE	Hatching % age	
Flubendiamide	LC_{10}	96 ±4	5	1.51±0.22
	LC_{25}			0.93±0.18
	LC_{50}			0.36±0
Spirotetramet	LC_{10}	3648 ±76	7	5.51±0.24
	LC_{25}	6771 ±182	11	3.07±0.22
	LC_{50}			
	Control	10709 ±101	93	9.52±0.26

MRGR = Growth Rate

SE= Standard Error

Growth rate= ln