**Studies on Dissipation Pattern and Bioefficacy of Combination Product, Spirotetramat 120 SC +Imidacloprid 120 SC against Onion thrips, *Thrips tabaci* Lindemanduring *Rabi* Season**

**Abstract**

Studies on bioefficacy of seven insecticides were evaluated by adopting good agriculture practices against onion thrips. First application was done as incidence of thrips was observed at 35 days after transplanting and subsequent applications were given at an interval of 10 days. Population of thrips was recorded on five randomly selected plants in each plot. While, for dissipation studies three applications of insecticide given at an interval of seven days starting at 45 days before harvesting @ 75 g a.i./ha (recommended dose) and 150 g a.i./ha (Double the recommended dose). QuChERs method used for residue analysis and quantification of samples by using High Performance Liquid Chromatography. Out of seven insecticides fipronil 30 a.i./ha proved to be most effective insecticide, also the combination of spirotetramat + imidacloprid was effective against onion thrips. In case of residue study the initial residues of spirotetramat (0.51 and 0.97 mg/kg) and imidacloprid (0.69 and 1.43 mg/kg) reached below quantification limit on 5th and 7th day at recommended and double the recommended doses, respectively. Half-life values for spirotetramat were 1.63 and 2.01 days while, for imidacloprid 1.63 and 1.56 days at recommended and double the recommended doses, respectively. Whereas, soil collected at harvest, did not record any residues of spirotetramat, spirotetramat-enol and imidacloprid at both the doses. Evaluating the assessment of insecticides, fipronil @ 30 g a.i./ha was proved to be highly effective by consistently suppressing the thrips population (3.26 thrips/plant & 86.62 % reduction over control). Considering LOQ of 0.05 mg/kg for spirotetramat and imidacloprid pre harvest interval (PHI) of seven days can be recommended for consumer safety.

**Keywords:** Residue, HPLC, spirotetramat, Imidacloprid, Onion, QuChERs

**Introduction**

Onion (*Allium cepa* L.) belongs to family Alliaceae is one of the most popular bulb vegetable originated from Central Asia (Brewester, 1994). It has special qualities which add taste and flavor to food as well as medicinal value. It is mainly used in India for cuisine and culinary preparation. Onion has therapeutic value and used as domestic remedy for scurvy. It has been used in dehydrated form or as preserved food product, hence there is an increasing demand for onion in food industries. It also has export, nutritional and medicinal values due to “allyl propyl disulphide” which is a volatile compound and acts as gastric stimulant. It is rich in flavonoids and alkenyl cysteine sulphoxides which help in preventing heart disease and other ailment to human being (Gareth *et al*. 2002). The crop attacked by number of pests among all, thrips are found to be the major pest and reported upto 50% crop losses (Nault and Shelton, 2012). Thrips are difficult to control due to their mobile stages and hide in leaf sheath, hence they escape from spray. Literature reveals that vegetables contain the residues of pesticides above their respective maximum residue limit (MRL) (Taneja, 2005) may pose health hazards to consumers (Elliion *et al*., 2000; Mukherjee and Gopal, 2003).

So, considering the economic importance of pest and crop value, studies were planned to study the bioefficacy of combination of spirotetramat 120 SC + imidacloprid 120 SC and also, studies on dissipation pattern of pesticides were conducted globally to assess the environmental load of their residues so as to recommend the safe waiting periods. The quantification of pesticide is done by Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) methods (Anastassiades, 2003).

**Materials and Methods**

**Bioefficacy:** In order to study the field bioefficacy of insecticides against onion thrips, five plants from each plot were selected randomly for recording the observations. Number of thrips present inner to leaf sheath of onion plants was recorded (Moraiet and Ansari, 2015) The observations were recorded at 1 day prior to spray treatment as precount and one, three, five and ten days after each spray consider as post count. First insecticides application was done as incidence of thrips observed at 35 days after transplanting and subsequent applications were given at an interval of 10 days.

**Dissipation:**

**Chemicals**

Acetonitrile (HPLC grade), Sodium sulphate anhydrous purified, Magnesium sulphate (Analytical Grade reagent), Acetone, Toluene, η- Hexane (HPLC grade) and PSA (Primary Secondary Amine).

**Sample collection**

Onion plant samples collected randomly from each replication after three applications at 0 hrs, 1, 3, 5, 7, 10 and 15 DAS. Soil samples collected at harvest.

**Extraction and Clean-up**

A representative 15 g of homogenized sample was taken in a clean 50 ml polypropylene tube and kept the filled tubes in deep freeze. To this, added 30 ml of acetonitrile (v/v), homogenized the sample @ 1400-1500 rpm for 2-3 min using heidolph silent crusher, added 3.0 g anhydrous sodium chloride, gently hand shake and centrifuged the content @ 3000 rpm for 3 min. Then transferred 16 ml supernatant to tube containing 9 g anhydrous magnesium sulphate, centrifuged and transferred 8 ml supernatant to 40 mg PSA and 1.2 g sodium sulphate (anhydrous) containing tube. It was shaken vigorously for 1.0 min (manually). Then centrifuged @ 3000 rpm for 5.0 min and

transferred aliquot of 2.0ml supernatant to the tube and evaporated to dryness to LC nitrogen and make up to 1.0 ml with acetonitrile and filter through 0.2 μ filter for HPLC analysis. (Anastassiades *et al.,* 2003).

**Residue Determination**

Residue analysis of spirotetramat and imidacloprid was performed using HPLC. Identification of insecticide residue was accomplished by retention time (RT) and compared with known standard (CRM) at same conditions. Quantitates were calculated on peak area basis by using following formula.

 Area of μl of sample conc. of

 sample injected standard (ppm)

 **Residues**  = X X X Final vol. (ml)

 (**mg kg**-**1**) Area of µl of standard wt. of sample

 Standard injected

 Sample wt. (g) X Aliquot taken (ml)

**Weight of sample(g)** =

 Volume of solvent added (ml)

Weigh 15 g homogenized sample in clean 50 ml polypropylene tube

Add 30 ml acetonitrile

Homogenized the sample at 1400-1500 rpm for 2-3 min using Heidolph silent crusher

Add 3 g sodium chloride

Handshakes gently and centrifuge the content at 3000 rpm for 3 min

Transfer 16 ml supernatant to the 50 ml tube containing 9 g anhydrous sodium sulphate

Transfer 8 ml extract to the 15 ml tube containing 40 mg PSA and 1.2 g anhydrous magnesium sulphate

Vortex for 30 sec and centrifuge the sample mixture at 3000 rpm for 5 min

Transfer 2 ml extract into test tube and evaporate to dryness using LC nitrogen

Reconstitute the volume with 1 ml acetonitrile Filter through 0.2 micron filter and fill the vials for HPLC injection.

**Chart 1. Extraction and cleanup of onion plant and soil sample**

**Method Validation**

Validation parameters *viz*., LOD, LOQ, linearity, accuracy and precision were determined before analysis of samples.

**Linearity**

A linearity study was performed to determine the performance of detector. Linearity curve was established with five linear concentrations of standard and corresponding peak area. The response was linear over the range tested and regression coefficient (R2) values were greater than 0.99 over the range tested

**Recovery**

Accuracy of the analytical method was determined by recovery studies. The analytical method employed for the extraction and cleanup of onion whole plant, dry bulb and soil sample was found accurate and precise as mean recovery percentage and relative standard deviation (RSD), were within its acceptable limits prescribed by SANCO (2011). According to SANCO (2011) guidelines, analytical method was record mean recovery standard deviation (RSD) below 20 per cent is accurate and precise.

**HPLC Analysis**

The analysis of samples of spirotetramat and imidacloprid residues was carried out with Shimadzu make high-performance liquid chromatography system equipped with photodiode array detector (HPLC) and quaternary pump (LC-20 AT). LC solution software was used as the data analysis system. The operating parameters of the instruments are shown below.

|  |  |
| --- | --- |
| **Column type** | Purospher @ STAR(Hibar) RP 18(5u) m-150-4.6 |
| **Mobile phase** | Acetonitrile : Water (80:20) |
| **Flow rate** | Spirotetramat : 0.80 ml min-1 |
| Spirotetramat- enol : 0.60 ml min-1 |
| Imidacloprid : 0.80 ml min-1 |
| **Wavelength** | Spirotetramat : 254 nm |
| Spirotetramat- enol: 210 nm |
| Imidacloprid : 272 nm |
| **Injector volume** | 20 µl |
| **Retention time** | Spirotetramat : 4.3 minSpirotetramat- enol : 3.5 minImidacloprid : 3.2 min |

**Table 1. Operating parameters of HPLC Analysis**

**Results and Discussion**

Pooled data obtained from field efficacy revealed that plot treated with fipronil @ 30 g a.i./ha (3.26 thrips/plant & 86.62 % reduction over control) whereas, spirotetramat + imidacloprid @ 75+75 g a.i./ha (6.50 thrips/plant& 73.33%) also recorded minimum incidence of thrips indicating their significance over other treatments.

Overall result of analysis of onion whole plant (immature bulb and leaves) after third application of spirotatramat 120 SC @ 75 and 150 g a.i.ha-1 (Table 2 & Fig 1.). The mean initial residue of spirotetramat was 0.51 and 0.93 mg kg-1. These residues were dissipated to the 0.43 and 0.87 mg kg-1 after one day at recommended dose and double the recommended dose, respectively. The present data showed that, there was loss of more than 50 % residue after 3rd day. The residues dissipated to 82.35% and 94.84% at 3rd and 5th day in both the doses of application. The residue of spirotetramat reached below its limit LOQ of 0.05 mg kg-1 on at 5th day at recommended dose and 7th day at double the recommended dose. Metabolite of spirotetramat *i.e.* spirotetramat-enol, was not detected in any of the sample. Half-life values calculated for spirotetramat on onion crop were 1.63 and 2.01 days, for recommended and double the recommended dose, respectively.

In case of imidacloprid 120 SC @ 75 and 150 g a.i.ha-1 the average initial residue of imidacloprid on onion plant were found to be 0.72 and 1.43 mg/kg, respectively. These residues dissipated to 0.59 and 0.78 mg/kg after a day at recommended and double the recommended doses, respectively. The residue dissipated to 89.02 and 95.80 per cent at 5th and 7th day in both the doses of application (Table 3 & Fig 2.). The residues of imidacloprid 89.02 and 95.80 reached below quantification limit (BQL) of 0.05 mg/kg after the 5th and 7th day of recommended and double the recommended doses, respectively. As regards to imidacloprid, half-life values calculated on onion crop were 1.63 and 1.56 days, for recommended and double the recommended doses, respectively.

Information on the residues of spirotetramat and imidacloprid on onion is not available. However, the present findings are in close agreement with Pandiselvi *et al.* (2010) who found that residues of spirotetramat in cotton plant, was below determination limit at 3rd and 5th day, respectively. Cotton seed, lint and oil samples collected at the time of harvest did not detectable residues of spirotetramat. Similar results were obtained by Mohapatra *et al*. (2012)who studied, the persistence of spirotetramat in mango fruits spirotetramat @ 90 and 180 g a.i.ha-1 and reported that the residues were found below determination limit at 10 days for both doses. Chahil *et al.* (2014) revealed the persistence of spirotetramat and imidacloprid in chilli fruits spirotetramat (12%) and imidacloprid (12%) @ 1000 and 2000 ml ha-1and reported that imidacloprid residues were found to be below its LOQ of 0.01 mg kg-1 at 7th and 10th day, respectively.

Analysis of soil sample was carried out on 30th day after third day. No residues were detected in untreated control samples. The residues of insecticides (*i.e*. spirotetramat, spirotetramat-enol and imidacloprid) in soil at harvest were found to be below quantification limit (BQL) at recommended and double the recommended doses, respectively. Shukla *et al*. (2016) and Vemuri *et al*. (2014) lend support to present findings.

**Conclusion**

It can be concluded that spirotetramat and imidacloprid persisted up to 5 to 7 days on onion at recommended and double the recommended doses, respectively. At present no MRL is prescribed for spirotetramat and imidacloprid in onion, considering LOQ of 0.05 mg/kg for spirotetramat and imidacloprid pre harvest interval (PHI) of seven days can be recommended to produce residue free onion. As per the effectiveness of combination of spirotetramat 120 SC + imidacloprid 120 SC can be recommended to control onion thrips.

**Graph 1 : Correlation matrix of Spirotetramat**

**Graph 2 : Correlation matrix of Imidacloprid**

**Graph 3 : Correlation matrix of Cis-enol**

 **Table 2. Dissipation of spirotetramat residues at different interval in onion plant (immature bulb and leaves) and soil**

|  |  |
| --- | --- |
| **Interval between last application and sampling** | **Residues mg kg-1** |
| **Untreated control** | **Spirotetramat @ 75 g a.i/ha** | **Spirotetramat @ 150 g a.i./ha** |
| **R1** | **R2** | **R3** | **Mean (S.D.)** | **R1** | **R2** | **R3** | **Mean (S.D.)** | **Dissipation****(%)** | **R1** | **R2** | **R3** | **Mean (S.D.)** | **Dissipation****(%)** |
| **0 Day** | ND | ND | ND | ND | 0.49 | 0.51 | 0.52 | 0.51(± 0.02) | - | 1.02 | 0.96 | 0.93 | 0.97(±0.05 ) | - |
| **1 Day** | ND | ND | ND | ND | 0.44 | 0.42 | 0.43 | 0.43(± 0.01) | 15.68% | 0.81 | 0.83 | 0.87 | 0.85(± 0.03) | 12.37% |
| **3 Day** | ND | ND | ND | ND | 0.24 | 0.25 | 0.25 | 0.25(± 0.01) | 50.98% | 0.40 | 0.38 | 0.41 | 0.40(± 0.02) | 58.76% |
| **5 Day** | ND | ND | ND | ND | 0.08 | 0.09 | 0.08 | 0.09(± 0.01) | 82.35% | 0.15 | 0.17 | 0.17 | 0.17(± 0.01) | 82.47% |
| **7 Day** | ND | ND | ND | ND | BQL | BQL | BQL | BQL | - | 0.051 | 0.052 | 0.051 | 0.05(± 0.001) | 94.84% |
| **10 Day** | ND | ND | ND | ND | BQL | BQL | BQL | BQL | - | BQL | BQL | BQL | BQL | - |
| **15 Day** | ND | ND | ND | ND | BQL | BQL | BQL | BQL | - | BQL | BQL | BQL | BQL | - |
| **Soil at harvest** | ND | ND | ND | ND | ND | ND | ND | ND | - | ND | ND | ND | ND | - |
| **DT50 (Days)** |  |  | **\_** |  |  | **1.63** | **2.01** |  |

 \*BQL= Below Quantification Limit (0.05 mg/kg) \*ND = Not Detected \*DT = Dissipation Time \*SD = Standard Deviation

**Table 3. Dissipation of imidacloprid residues at different interval in onion whole plant (immature bulb and leaves) and soil**

|  |  |
| --- | --- |
| **Interval****between last****application****and sampling** | **Residues mg kg-1** |
| **Untreated control** | **Imidacloprid @ 75 g a.i/ha** | **Imidacloprid @ 150 g a.i./ha** |
|  | **R1** | **R2** | **R3** | **Mean (S.D.)** | **R1** | **R2** | **R3** | **Mean (S.D.)** | **Dissipation****(%)** | **R1** | **R2** | **R3** | **Mean (S.D.)** | **Dissipation****(%)** |
| **0 Day** | ND | ND | ND | ND | 0.72 | 0.73 | 0.69 | 0.72(±0.02) | - | 1.44 | 1.52 | 1.32 | 1.43(± 0.10) | - |
| **1 Day** | ND | ND | ND | ND | 0.58 | 0.57 | 0.61 | 0.59(±0.02) | 18.05% | 0.77 | 0.77 | 0.78 | 0.78(± 0.01) | 45.45% |
| **3 Day** | ND | ND | ND | ND | 0.39 | 0.38 | 0.37 | 0.38(±0.01) | 47.22% | 0.63 | 0.62 | 0.60 | 0.62(±0.02 ) | 56.64% |
| **5 Day** | ND | ND | ND | ND | 0.087 | 0.079 | 0.084 | 0.08(±0.004) | 89.02% | 0.15 | 0.14 | 0.15 | 0.15(±0.01 ) | 89.51% |
| **7 Day** | ND | ND | ND | ND | BQL | BQL | BQL | BQL | - | 0.065 | 0.058 | 0.067 | 0.06(± 0.005) | 95.80% |
| **10 Day** | ND | ND | ND | ND | BQL | BQL | BQL | BQL | - | BQL | BQL | BQL | BQL | - |
| **15 Day** | ND | ND | ND | ND | BQL | BQL | BQL | BQL | - | BQL | BQL | BQL | BQL | - |
| **Soil at harvest** | ND | ND | ND | ND | ND | ND | ND | ND | - | ND | ND | ND | ND | - |
| **DT50 (Days)** | - | **1.63** | **1.56** |

\*BQL= Below Quantification Limit (0.05 mg/kg) \*ND = Not Detected \*DT = Dissipation Time \*SD = Standard Deviation

**Linearity of spirotetramat, spirotetramat-enol and imidacloprid**

**Fig. 1. Dissipation pattern of spirotetramat in onion**

**Fig. 2. Dissipation pattern of imidacloprid in onion**

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