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Original article

In-vitro and *In-vivo* management of *Meloidogyne incognita* (Kofoid and White) Chitwood and *Rhizoctonia bataticola* (Taub.) Butler in cotton using organic's

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ABSTRACT

Root-knot nematodes *Meloidogyne incognita* (Kofoid and White) Chitwood and *Rhizoctonia bataticola* (Taub.) Butler, fungus, are very dangerous root damaging pathogens. Present study was planned to establish a chemical control of these root deteriorating pathogens under lab conditions as well as in field. Maximum death rate of nematode juveniles and minimum numbers of nematode eggs hatched were recorded in plates treated with Cadusafos (Rugby® 100G) @12 g/100 ml and Cartap® (4% G) @9g/100 ml. Chemical treatment of *Rhizoctonia bataticola* with Trifloxystrobin + Tebuconazole (Nativo®) @0.2 g/100 ml and Mancozeb + Matalaxyl (Axiom) @0.25 g/100 ml significantly controlled the mycelial growth in plates. The best treatments tested in laboratory were applied in field as protective and curative treatments. Results proved that chemical control of root-knot nematode and root rot fungi by tested chemicals at recommended time and dose is a significant management technique under field conditions.

1. Introduction

Sedentary endoparasitic plant parasitic nematodes (PPNs) of genus *Meloidogyne* are highy polyphagous and root deteriorating agents of various crops worldwide (Jones et al., 2013; Mantelin et al., 2017). Among PPNs, *M. incognita* is economically important with high rate of reproduction and broad host range (Abad & Williamson 2010). Second stage juvenile (J_2) attacks vascular bundles in plants and produce multinucleated giant cells for uninterrupted supply of nutrients (Vovlas, 2005, Jones, 2011). Interaction of PPNs with fungi has been numerously reported by several scientists in different crops (Bond et al., 2004; Back et al., 2006; Khan & Haque, 2013). The most damaging and catastrophic plant parasitic nematodes belong to genus *Meloidogyne* especially *M. incognita* having more than 90 species (Taylor & Sasser, 1978; Hunt and Manzanilla-López, 2005). In Pakistan, various researchers have

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reported root-knot nematode parasitizing ornamental plants, field crops and vegetable crops (Abad et al., 2003; Charchar & de Aragão, 2005; Khan et al., 2006). Among fungi *Pythium* species, *Verticillium* species, *Fusarium* species, *Phytophthora* species and *Rhizoctonia* species are frequently observed causing root rot diseases (Khan et al., 2017). Root rot in cotton caused by the interaction of nematodes (*M. incognita*) and fungi (*R. bataticola* Taub. Butler) cause maximum losses and are difficult to control (Khan et al., 2017).

Use of synthetic chemicals to manage the pests is considered effective as compared to all other practices like bio-control agents, resistant varieties and cultural controls (Barker & Koenning, 1998). Various synthetic chemical (i.e. Cadusafos, Diflubenzuron, Carbofuran, Thiocarbamate etc.) and fungicides (i.e. Trifloxystrobin, Tebuconazole, Fluopyram etc.) have been evaluated by several researchers against root-knot nematode and root rot fungi (Kamunya et al., 2008; Ruan et al., 2009; Safdar et al., 2012; Lashein et al., 2014; Patón et al., 2017) under field and lab conditions. Chemical evaluation against both pathogens, *M. incognita* and *R. bataticola*, isolated from cotton is not well documented in literature. The present study was planned to accomplish three purposes; to check the efficacy of ten synthetic chemicals against root-knot nematode; to reduce mycelial growth of root rot fungi using ten fungicides under *in vitro* trials and to evaluate both fungicides and nematicides as protective and curative measures against disease complex of cotton under field conditions.

2. Materials and methods

Root pathogens i.e. *M. incognita* and *R. bataticola* were isolated from the diseased samples taken during the research from cotton fields. The research was conducted during months August and September in year 2017. Infected and Healthy root samples along with soil were taken in polythene bags (15 × 20 cm) and stored in the cooler box. The samples were taken in the laboratory and processed for the isolation, purification and identification of pathogens.

2.1. In vitro management

2.1.1. Nematodes

For isolation of nematodes from cotton, roots were isolated from the soil and then washed and weighed. Whitehead and Hemming tray method and Baermann funnel method was practiced for nematode isolations from soil and root samples respectively (Whitehead & Hemming, 1965) whereas for egg hatching the entire root system was chopped carefully and placed in a mist chamber for 5 days (McKenry & Roberts, 1985). After nematode isolation the identification of nematode was done by making parineal patterns of mature females (Jepson, 1987). At least 10 parineal patterns were examined for identification under a stereomicroscope (Olympus SZ 61) at 3.5X magnification (Eisenback et al., 1981). To get regular supply of inoculum for experiments, mass culturing of root-knot nematodes was done on the susceptible tomato variety i.e. Money maker. Three concentrations i.e. recommended ®, half (R/2) and quarter (R/4) of each synthetic chemical were prepared according to recommended dose by adding requisite amount of water against egg hatching and juvenile mortality of *M. incognita*. Freshly hatched juveniles, within 48 h, of root-knot nematode were used in mortality test 50 µl suspension was placed in each petri dish containing 80 *M. incognita* juveniles (J_{2s}). Each treatment was replicated five times. Data were recorded after 24, 48 and 72 h. Juvenile mortality was calculated and corrected by Abbot's formula (Abbott, 1925).

$$Mortality\ (\%) = \frac{t - c}{100 - c} \times 100$$

The juveniles were considered dead if they do not move by probing with a fine needle (Abbasi *et al.,* 2008) and if they move and appeared winding shape they considered alive (El-Rokiek & El-Nagdi, 2011).

For hatching test, Hussy & Barker (1973) method was used for the isolation of *M. incognita* eggs. Three concentration of each chemical were added in each petri dish contained 250 eggs. Each treatment was replicated five times and incubated at 25 °C ± 2. Design used for mortality and hatching test was completely randomized design. Data were recorded after 24, 48 and 72 h. Percent egg hatching was calculated and corrected by Abbot's formula. After each count the eggs were washed with distilled water and transferred to fresh concentration of chemicals.

2.1.2. Fungus

Samples were taken in laboratory and roots were cut into small pieces (5–6 cm), washed to clean and dipped in 2% sodium hypochlorite for two minutes for disinfestation. Post washing with distilled water was done twice and samples were placed on sterilized filter paper for drying. Segments were plated on potato dextrose agar (PDA) for isolation of suspected fungus. All the plates were incubated at 28 ± 2 °C for 5–7 days for recovery of pathogen (Sharma et al., 2012). On basis of morphological characters the fungus was identified and examined under dissecting microscope (Ellis, 1971). Purification of root rot fungi (*R. bataticola*) was done for further experiments. Fungicides were weighed and dissolved in 5 ml distilled water and diluted upto 100 ml. Poison food technique was followed by pouring potato dextrose agar (into 9 cm petri dishes. Five replications of each fungicide with each concentration were made including control (without fungicide) under complete randomized design. Ten days old *R. bataticola* was inoculated using sterilized inoculating needle. Petri dishes were labeled and placed in incubator at 28 ± 2 °C. Radial mycelial growth was measured after five and ten days interval (Mamza et al., 2010).

2.2. In vivo management

In situ, three plots were maintained to study the effect of pathogens on cotton yield. In first plot the cotton was grown under natural environmental conditions (control treatment) without any application of synthetic chemicals and fungicide for root pathogens. Cadusafos (Rugby® 100G) and Trifloxystrobin + Tebuconazole (Nativo®) were applied in second plot (as protective treatment) and third plot (as curative treatment). Treatments in second plot were applied two times, first in month of June and second in month of August, in one cropping season whereas as in third plot single application of chemicals, in month of August, was practiced using randomized complete block design. Sowing of cotton was done on 28-04-2018. Susceptible cotton variety (CRIS-134) was selected after screening of different germplasm to check the efficacy of chemicals. Dimension of each experimental plot was 272ft^2 and plant population was maintained at 145 plants per plot. Row to row distance was maintained at 2.5ft whereas plant to plant distance was 0.75ft. All agronomic practices (preparation of land, irrigation, fertilizers, hoeing etc.) and insecticides application was done for the better growth of cotton plants. Data regarding disease incidence of control, protective treatment and curative treatment were collected after 45 days, 90 days, 120 days and 150 days after sowing whereas yield was calculated at end of season.

2.3. Statistical analysis

Means and standard errors were calculated in Microsoft excel worksheet 2010. Statistical analysis was done using Statistix 8.1 and Statistical Analysis System (SAS) 9.3 software's. Data were analyzed under two factor factorial arrangement. Factors were

fungicide doses and chemicals. Treatment means were separated at 5% significant level (Steel & Torrie 1980).

3. Results

3.1. Evaluation of fungicides against Rhizoctonia bataticola

Ten fungicides namely Thiophenate methyl (Thiophenate methyl), Triger (Tebuconazole), Shelter (Difinoconazole), Efogan (Pyrazophos), Derosal (Carbendazim), Axiom (Mancozeb + Mata laxyl), Vampire (Propiconazole), Reflex (Difinoconazole + Propico nazole), Nativo® (Trifloxystrobin + Tebuconazole) and Hombre (Imidacloprid + Tebuconazole) were evaluated against *R. bataticola* at three (R, R/2 and R/4) concentrations. A control without any treatment was also maintained for the comparison. At recommended concentration Nativo® (1.9) and Axiom (2.6) showed significant results whereas Thiophenate methyl (3.57) shown minimum results as compared to all treatments applied as shown in Fig. 1. Both Nativo® and Axiom were significantly effective fungicides used against *R. bataticola* at R/2 and R/4 concentrations. Results showed that among all concentrations used the recommended dose was effective whereas Nativo® and Axiom were most effective against pathogen. Results were significantly different from each other (P = 0.05) as shown in Fig. 1.

3.2. Evaluation of synthetic chemicals against juvenile mortality of M. incognita at R, R/2 and R/4 concentrations

Ten synthetic chemicals namely Rugby® (Cadusafos), Movento® (Spirotetramat), Cartap® (Thiocarbamate), Regent® (Fipronil), Steward® (Indoxacarb), Vimax™ (Acetamiprid), Virtako® (Thiamethoxam + chlorantraniliprole), Arrivo™ (Cypermethrin), Actara® (Thiamethoxam) and Silk™ (Bifenthrin) were evaluated against J_{2s} of *M. incogbnita*. Mortality percentage of juveniles were calculated at R concentration after 24, 48 and 72 h. At R concentration, Maximum juvenile mortality after 24 h, 46.4% and 43.2%, after 48 h, 81.4% and 77.8% and after 72 h, 85% and 80.2%, was caused by Rugby® and Cartap® respectively Fig. 2. At R/2 concentration, maximum juvenile mortality was shown by Rugby® (31%) and Cartap® (38.2%) after 24 h whereas after 48 h results were 57.4% and 72.4% respectively. After 72 h Rugby® exhibited (79.2%) juvenile mortality whereas Cartap® indicated (73.8%) juvenile mortality Fig. 3. At R/4 concentration, after 24 h maximum juvenile mortality was shown by Cartap® (28.2%) and Rugby® (23.2%). After 48 h Cartap® showed (62.4%) juvenile mortality whereas mortality shown by Rugby® was (47.4%). Results represented that after 72 h Rugby® gave (71%) juvenile mortality whereas Cartap® showed (65.6%). All results were compared with control. The results were significantly different from each other (P = 0.05) Fig. 4.

3.3. Evaluation of synthetic chemicals against egg hatching of M. incognita at R, R/2 and R/4 concentration

Chemicals were also evaluated against nematode egg hatching. Maximum inhibition was recorded by Rugby® (46.4%) and Cartap® (43.2%) after 24 h, after 48 h, 81.4% and 77.8%, and after 72 h, 85% and 80.2% respectively Fig. 5. At R/2 concentration Rugby® (43.8%) and Cartap® (39.2%) gave best results after 24 h whereas after 48 hresults were 74.6% and 69.4%. After 72 h Rugby® revealed (78.4%) whereas Cartap® indicated (73%) egg inhibition Fig. 6. At R/4 concentration, Maximum egg inhibition after 24 h was shown by Rugby® (39.4%) and Cartap® (32.4%) whereas after 48 h results indicated that significant egg hatching inhibition was shown by Rugby® (69.4%) and Cartap® (64.2%) Fig. 7. After 72 h Rugby® (72.4%) and Cartap® (68.6%) presented significant results by inhibiting egg hatching. The results were significantly different from each other (P = 0.05).

3.4. Evaluation of Cadusafos (Rugby®) and Trifloxystrobin + Tebuconazole (Nativo®) in field

Rugby® and Nativo® showed significant results under field conditions against *M. incognita* and *R. bataticola*. After 45 days no disease incidence was calculated in all treatments including control.

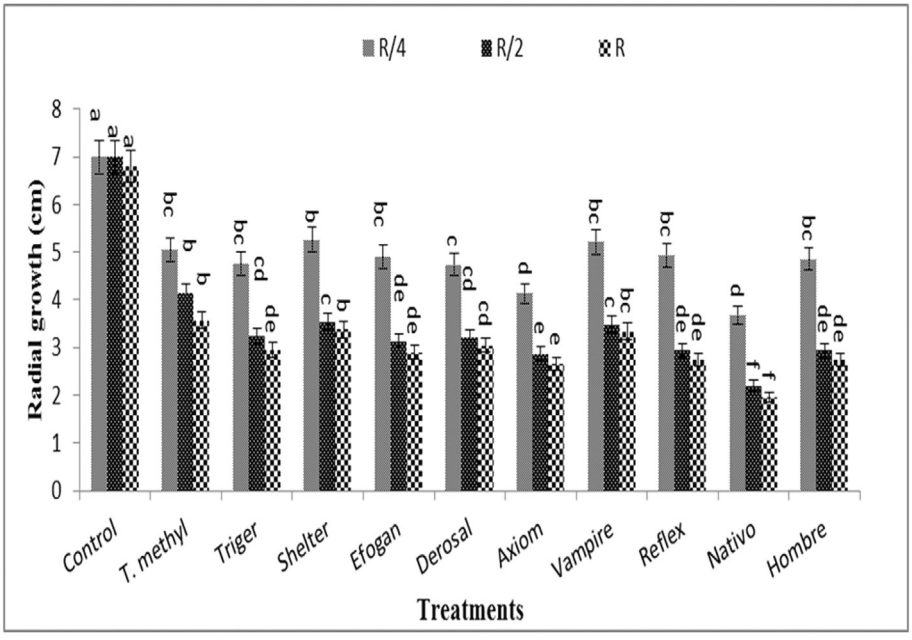

Fig. 1. Evaluation of fungicides at Recommended (R), Half (R/2) and Quarter (R/4) of recommended concentrations against *Rhizoctonia bataticola*. Vertical bars represents +S.E of means. Different letters at same treatment bars shows significant difference at P ≤ 0.05.

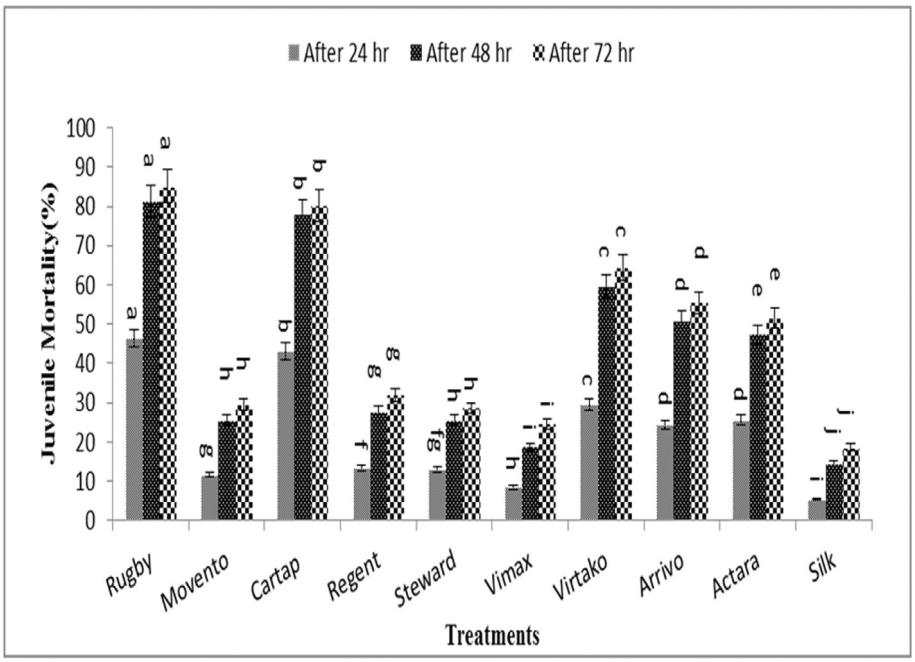

Fig. 2. Evaluation of synthetic chemicals against Juvenile mortality of *Meloidogyne incognita* at Recommended (R) concentration. Vertical bars represents +S.E of means. Different letters at same treatment bars shows significant difference at $P \leq 0.05$.

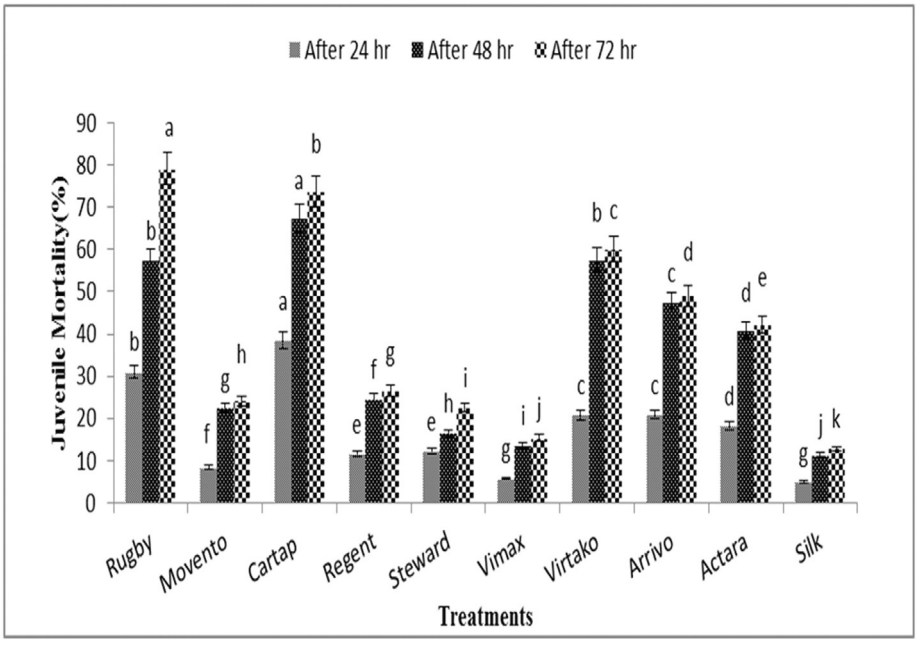

Fig. 3. Evaluation of synthetic chemicals against Juvenile mortality of *Meloidogyne incognita* at Half (R/2) of recommended concentration. Vertical bars represents +S.E of means. Different letters at same treatment bars shows significant difference at $P \leq 0.05$.

In protective treatment plot the application of synthetic chemicals was applied before 90 days so no disease incidence was calculated whereas in curative treatment plot and control plot the disease incidence was 12.33% and 18.66% respectively after 90 days. Before third data collection the protective treatment plot was treated twice with synthetic chemicals whereas in curative treatment plot the chemicals were applied first time. Data was collected after 120 days showed 2.33% disease incidence in protective treatment plot whereas in curative treatment plot the disease incidence was 22.33% as compared to control (39.66%). After 150 days results showed minimum disease incidence (4.66%) in protective treatment plot whereas in curative treatment plot 38.33% disease incidence was noted as compared to control (67.33%) as shown in Fig. 8. Yield (kg) was also calculated to check the efficacy of synthetic chemicals applied to different treatments against pathogens. Maximum yield was calculated in protective treatment 3.42 kg/100 plants whereas in curative treatment yield was 2.51 kg/100 plants as compared to control (1.45 kg/100 plants) where no synthetic chemicals were applied. The results were significantly different from each other ($P = 0.05$) Fig. 9.

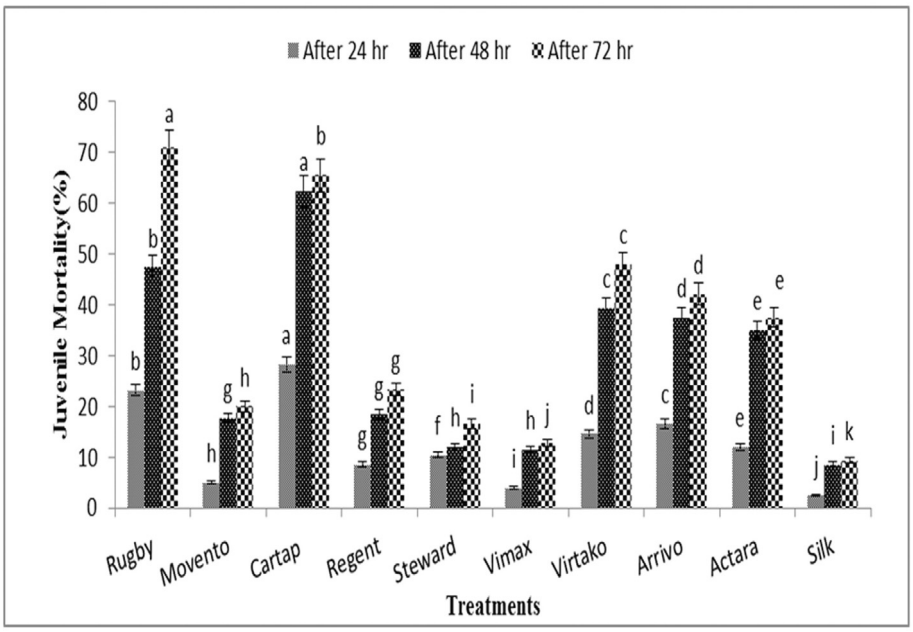

Fig. 4. Evaluation of synthetic chemicals against Juvenile mortality of *Meloidogyne incognita* at Quarter (R/4) of recommended concentration. Vertical bars represents +S.E of means. Different letters at same treatment bars shows significant difference at $P \leq 0.05$.

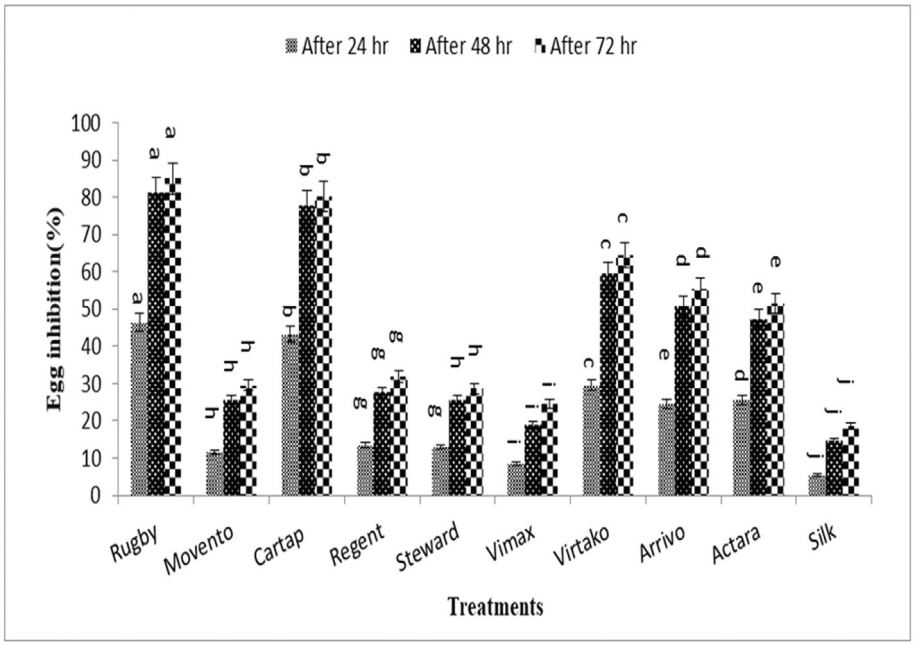

Fig. 5. Evaluation of synthetic chemicals against egg hatching of *Meloidogyne incognita* at Recommended (R) concentration. Vertical bars represents +S.E of means. Different letters at same treatment bars shows significant difference at $P \leq 0.05$.

4. Discussion

Since plants were first domesticated the farmers have been at the mercy of plant diseases. Uses of synthetic chemicals against plant pathogens have repeatedly altered the way crops are grown. Khan et al., 2017 reported disease complex in cotton, caused by *R. bataticola* and *M. incognita*, a very prevalent and destructive disease in cotton growing areas of Punjab, Pakistan. The disease spreads in July to September with symptoms of complete and sudden wilting in plants that causes maximum loss to cotton (Akhtar, 1972). Present research was planned to manage *R. bataticola* and *M. incognita* in cotton through synthetic chemicals and fungicides under *in vitro* and *in vivo* conditions. In vitro efficacy of ten fungicides was evaluated against *R. bataticola*, among all fungicides Nativo® and Axiom showed maximum inhibition of pathogen at all concentrations whereas best results were calculated at recommended dose (R). Similar *in vitro* studies have been conducted by many researchers to check the efficacy of fungicides with significant findings (Edington and Barron, 1971; Wong & Wilcox, 2001; Pérez et al., 2002; Parmar et al., 2017).

Under field conditions Nativo® and Rugby® were applied as protective and curative treatments and yield was calculated. Maxi-

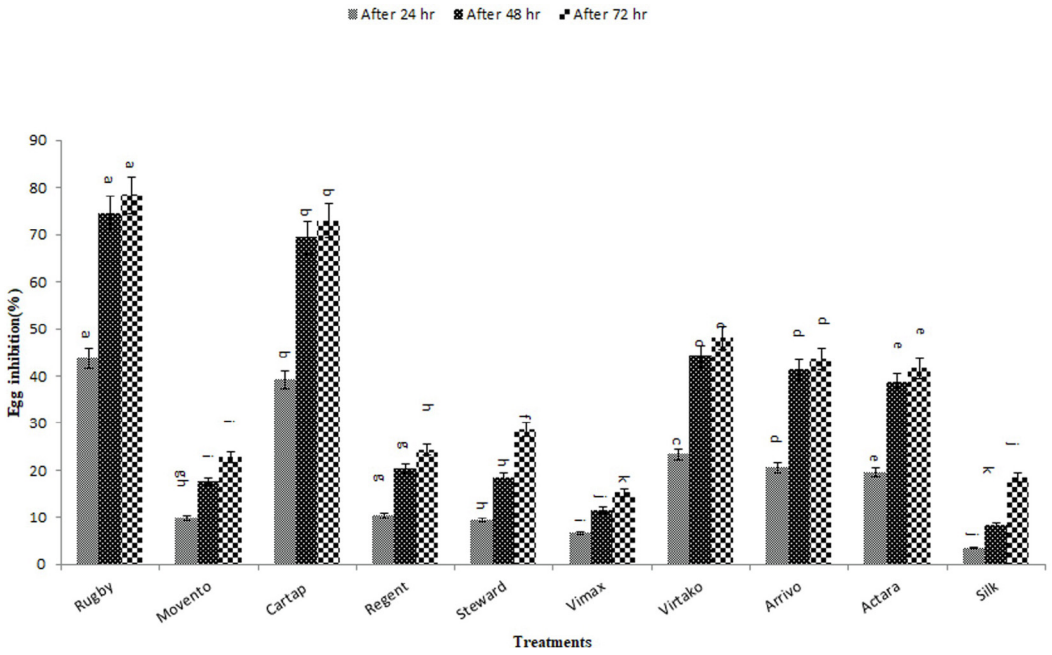

Fig. 6. Evaluation of synthetic chemicals against egg hatching of *Meloidogyne incognita* at Half (R/2) of recommended concentration. Vertical bars represents +S.E of means. Different letters at same treatment bars shows significant difference at $P \leq 0.05$.

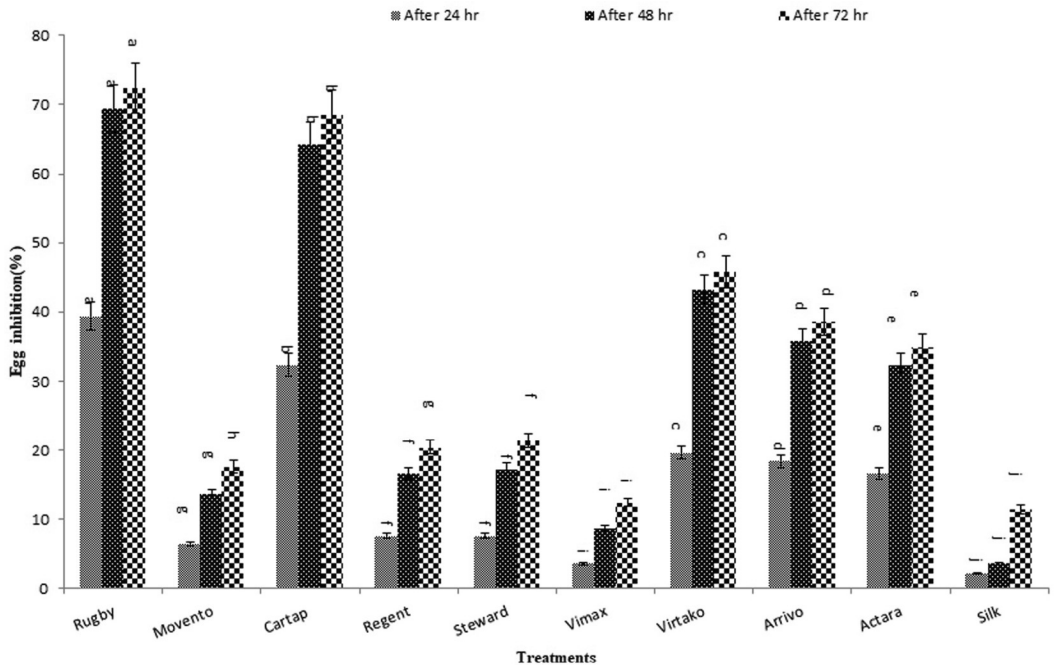

Fig. 7. Evaluation of synthetic chemicals against egg hatching of *Meloidogyne incognita* at Quarter (R/4) of recommended concentration. Vertical bars represents +S.E of means. Different letters at same treatment bars shows significant difference at $P \leq 0.05$.

mum yield was noted in protective treatment than curative treatment and nematode population was reduced effectively. Our findings are in line with those of (Husain & Masood, 1975; Stephan et al., 1998; Kim et al., 2002; Singh & Dabur, 2004; Rehman et al., 2006; Meher et al., 2010; Safdar et al., 2012). Khaliq et al., 2020 reported that used of fungicides against *R. bataticola* significatly reduced the disease severity and increased yield under filed condition in chickpea. Nativo® has active ingredient Trifloxystrobin + Tebuconazole belongs to inhibitors of cytochrome bc 1 (strobilurins) and inhibitor of sterol biosynthesis (triazoles) whereas Axiom has active ingredient Mancozeb + Matalaxyl belongs to Dithiocarbamate and Anilides group of fungicides respectively (Morton & Staub, 2008; Yang et al., 2011). Trifloxystrobin is a popular fungicide because of its versatility at controlling disease from different taxonomic classes, however, strobilurin enhances plant greening and improves yield (Gullino et al., 2000; Bartlett et al., 2002; Balba, 2007). Triazole fungicides are systemic and curative fungicides that cause leaves to be greener and improve yield depending on the crop (Buchenauer, 1995; Pernak et al., 2015). Soil and seed application of Matalaxyl effectively con-

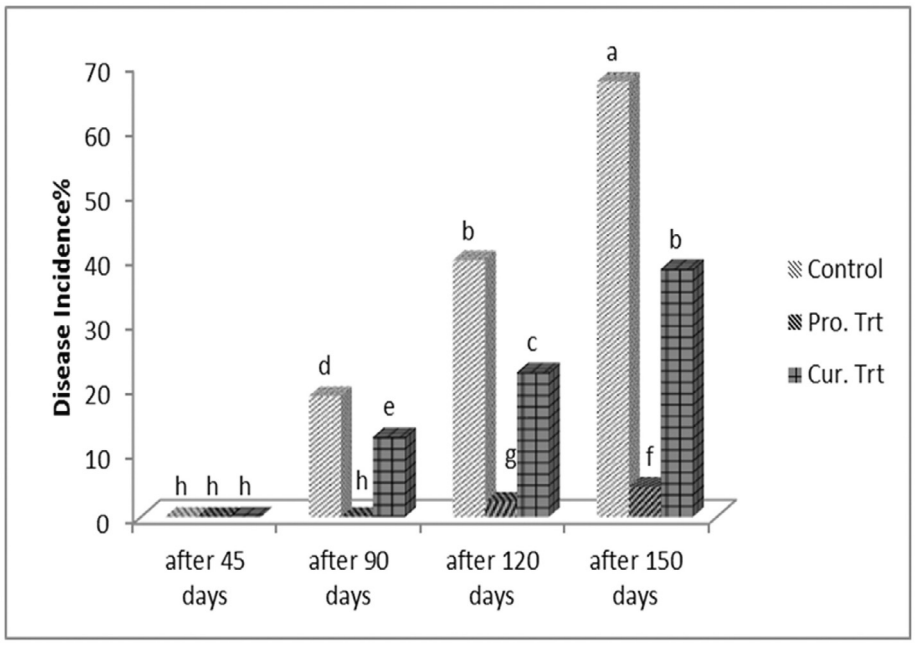

Fig. 8. Disease incidence (%) in Protective, curative and control treatments after 45, 90, 120 and 150 days. Vertical bars represents +S.E of means. Different letters at same treatment bars shows significant difference at $P \leq 0.05$.

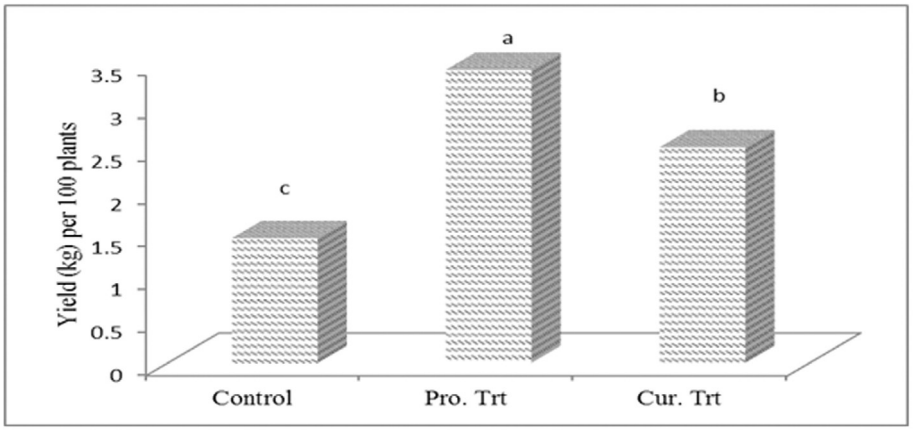

Fig. 9. Yield (kg) of cotton plants in Protective, curative and control treatments. Vertical bars represents +S.E of means. Different letters at same treatment bars shows significant difference at $P \leq 0.05$.

trols pythium and root infecting pathogen species (Klittich, 2008). Among synthetic chemicals, Cadusafos (Rugby® 100G) and Cartap® (4% G) gave maximum juvenile mortality and egg hatching inhibition at recommended dose. Cadusafos is a broad spectrum nematicide and effective against all nematodes particularly most destructive widespread nematodes in Pakistan *Meloidogyne* and *Globodera*. Cartap® and Cadusafos have ovicidal activity and J_2 mortality also recorded at 1% concentration (Nordmeyer et al., 1982; Nordmeyer & Dickson, 1985). Cadusafos was observed to reduce nematode juveniles and eggs hatching inhibition and our results are in conformity with Safdar et al., 2012. Koenning et al., 2004 reported nematicides a good source to control nematodes and their use enhances production. Radwan et al., 2012 evaluated various chemicals against *M. incognita* and found cadusafos effective causing, 86.63%, J_2 mortality whereas fosthiazate had highest nematicidal activity with 96.45% J_2 mortality. Organophosphate (cadusafos) and carbamate (Cartap®) compounds inhibit acetylcholinesterase (ACHE) at cholinergic synapses in nematode nervous system and affect the orientation behavior of nematodes (Wright, 1981; Opperman & Chang, 1990). Nelmes et al., (1973) suggested that these chemicals stamp down neuromuscular activity of nematodes and reduce their movement, invasion, rate of development and reproduction. Egg hatching inhibition, movement in soil and development of second stage juveniles in roots significantly suppressed by the application of organophosphate (cadusafos) and carbamate (Cartap®) compounds (Bunt, 1987; Takagi et al., 2020). The present study is helpful in mitigation of root infecting fungi and nematodes and their interactive study, in future, may provide better understanding for developing integrated disease management model for both pathogens.

5. Conclusions

Cadusafos (Rugby®100G) and Cartap® (4% G) are most effective nematicides, at 12 g/100 ml and 9 g/100 ml, whereas Nativo® (Trifloxystrobin + Tebuconazole) and Axiom (Mancozeb + Mata laxyl) are most efficient fungicides, at 0.2 g/100 ml and

0.25 g/100 ml, respectively whereas all concentrations significantly reduced egg hatching, nematode mortality and fungal growth. So, it can be concluded that combination of Rugby® with Nativo® can be successfully used as protective treatment for the management of *M. incognita* (Kofoid and White) chitwood and *R. bataticola* (Taub.) under field conditions for better yield and production in cotton.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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