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RESEARCH ARTICLE

EXPLORING THE EFFICACY OF CNSL-BASED BOTANICAL PESTICIDES FOR THE MANAGEMENT OF PESTS OF BHINDI (ABELMOSCHUS ESCULENTUS)

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Abstract

The consistent use of chemical pesticides over the years has led to various issues. To mitigate these problems, botanical insecticides have been used as a better alternative. One such botanical insecticide is cashew nut shell liquid (CNSL 20% EC), which contains a mixture of phenolic compounds. Under laboratory conditions, this insecticide was assessed against *Sylepta derogata* larvae in different concentrations ranging from 0.1 to 1, along with a chemical control. At 5, 7, and 10 DAT, CNSL 1% was found to be the most effective treatment, with 90% mortality recorded. A probit analysis was conducted to determine the effective dose, which was found to be 1%. The CNSL 20% EC was modified to produce two combination products, EC1 and EC2, by substituting half of the active ingredient with neem oil and pongam oil respectively, without changing other ingredients and their proportion. The developed formulations were evaluated for product stability and emulsion properties. A pot culture experiment was conducted on bhindi crops once during the vegetative and reproductive phases. At 7 DAT, EC1 @ 1% and CNSL 20% EC @ 1% caused over 95% reduction of sucking pests such as *Amrasca biguttula biguttula* and *Aphis gossypi* compared to the control. However, CNSL 20% EC produced only a 76.48% reduction of the chewing pest S. derogata, whereas treatments with EC1 and EC2 showed over 80% reduction. Sucking pests were effectively reduced (by more than 90%) by the application of EC2 as well. It was concluded that the combination products EC1 and EC2 were more effective in controlling pests than CNSL alone.

Introduction:-

Bhindi, *Abelmoschus esculentus* Moench. is one of the most widely utilized vegetable crops of the family Malvaceae (Saifullah and Rabbani, 2009) and is called “a perfect villager’s vegetable” because of its robust nature, dietary fiber, and distinct seed protein balance of both lysine and tryptophan amino acids (unlike the proteins of cereals and pulses) (Sanjeet et al., 2010).

Though a robust and resilient vegetable crop, Bhindi is susceptible to many insect pests. About 72 insect species have been reported to attack bhindi viz., leaf hopper, *Amrasca biguttula biguttula* (Ishida); aphid, *Aphis gossypii* (Glover); whitefly, *Bemisia tabaci* (Genn.); shoot and fruit borer, *Earias insulana* (Boisd.) and *E. vittella* (Fab.); leaf

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roller, *Sylepta derogata* (Fab.); red cotton bug, *Dysdercus koenigii* (Fab.); mite, *Tetranychus cinnabarinus* (Boisd.); green plant bug, *Nezara viridula* (Linn.); blister beetle, *Mylabris pustulata* (Thunb.); and green semilooper, *Anomis flava* (Fab.) (Nagar et al., 2017).

Farmers often use chemical pesticides to manage insect pests in vegetable crops. However, this can lead to high levels of insecticide residues in the produce, causing health problems for consumers.A remedy for this dilemma is the use of botanical pesticides.

Botanical pesticides are efficacious in managing different crop pests, are less expensive, easily biodegraded, have varied modes of action, their sources are easily available and have low toxicity to non-target organisms. Their varied modes of action are attributed to the phytochemical composition of different plants. Therefore, they can be incorporated into integrated pest management systems and contribute to sustainable agricultural production (Lengai et al., 2020).

The beauty of these compounds is that they are often easily decomposed by various common microbes in most soils. Consequently, the potential for environmental contamination is reduced (Khater, 2012).

Cashew plants, *Anacardium occidentale*, produce and store abundant phenolic compounds in their nut shell to protect their propagule from herbivores. This Cashew Nut Shell Liquid (CNSL) is one such material obtained in large quantities as a by-product of cashew processing industry. The technical CNSL obtained from the cashew industry after processing contains 62.86 per cent cardanol, 11.25 per cent cardol and 23.8 per cent polymeric material (Lubic and Thachil, 2003). Naturally occurring pericarp liquid is a mixture of phenolic compounds such as anacardic acid, cardol, cardanol and methyl cardanol (Venmalar and Nagaveni, 2005).

The pesticidal efficacy of CNSL was proved against chewing pests like Leucopholis coneophora Burmeister (John et al., 2008) as well as sucking pests like *Aphis craccivora* Koch (Olotuah and Ofuya, 2010) and *A. gossypii*, *Scirtothrips dorsalis* Hood. and *Polyphagotarsonemus latus* (Banks) (Sundaran, 2018). Mahapatro (2011) reported the insecticidal activity of CNSL (1 %) by stomach poisoning in diet incorporation on *Helicoverpa armigera* Hubner and *Spilarctia obliqua* Walker. CNSL emulsion 0.2 per cent was reported to cause 100 per cent mortality of chilli aphid A. gossypi 72 hours after treatment under laboratory conditions (Sundaran and Faizal, 2018). Two emulsifiable concentrate formulations (CNSL 20% EC and CNSL 20% + Pongam oil 20% EC) were developed at the Department of Agricultural Entomology, College of Agriculture, Vellayani and found effective against *A. craccivora* and *Riptortus pedestris* (Fabricius) at the rate of 0.3 per cent (Lekha, 2020).

The present project is envisaged to check the utility of this formulation with suitable modifications for the management of pests of bhindi.

Material and Methods:-

The insecticidal properties of an emulsifiable concentrate formulation of Cashew Nut Shell Liquid (CNSL 20% EC) developed in the Department of Entomology, College of Agriculture, Vellayani was evaluated against major pests of bhindi at the Department of Entomology, College of Agriculture, Vellayani. As the first step, different concentrations of CNSL viz., 0.1, 0.25, 0.5, 0.75 and 1per cent, prepared from the CNSL 20 % EC formulation were evaluated against the test insect, bhindi leaf roller (*S. derogata*), under laboratory conditions.

Maintenance of test insect

Field-collected *S. derogata* larvae were reared on fresh bhindi leaves in a plastic cover. Adult S. derogata were released onto a container with muslin cloth for mating and egg laying. They were provided with 20% honey solution on cotton wool as diet and a folded paper to lay eggs. The larvae upon hatching were transferred to tender bhindi leaves and reared with fresh leaves. Early third instar larvae from eggs of the same age were used for the experiment.

Preparation of cashew nut shell liquid 20 % EC

The CNSL 20% EC is a mixture of 20 per cent active ingredient (CNSL), 12 per cent emulsifier (blend of sodium oleate and span 20 in the ratio 91:9), 53% solvent (isopropyl alcohol), 5 per cent co-solvent (cyclohaxanone) and 10 per cent water (Lekha, 2020). The active ingredient is CNSL, the by-product of drum roasting method of cashew, purchased from Mahatma Cashew experts, Kollam, Kerala. To obtain 1 L of the formulation 109.2 g of sodium oleate was dissolved in 100 ml of sterile distilled water by placing it in the water bath at 50^0C. After dissolution, it was mixed with 10.8 ml of span 20 and well shaken with the help of mechanical shaker for 30 minutes. 200 ml of CNSL was mixed with 530 ml isopropyl alcohol and 50 ml cyclohexanone and shaken in mechanical shaker for 30 minutes separately. The above two mixtures were slowly blended together and well mixed in a mechanical shaker for about 45 minutes to obtain the final formulation.

Evaluation of CNSL 20% EC against test insect

Five different concentrations of CNSL viz., 0.1%, 0.25%, 0.50%, 0.75% and 1% were prepared and was tested against early third instar larvae of *S. derogata* derived from the stock culture (ten numbers per replication) in completely randomized block design with seven treatments and three replications. The treatments were applied uniformly to bhindi leaf harboring one larva using a spray bottle and then they were transferred to polyvinyl containers covered with muslin cloth. Leaves harboring larva treated with water serve as untreated check. The observations were taken at 1, 3, 5, 7 and 10 days after treatment.

Modification of CNSL 20% EC into combination products.

CNSL 20% EC was modified into two combination products of botanicals by substituting half of the active ingredient with other plant derived oils having insecticidal property. In the present study plant derived oils namely neem oil and pongam oil was used to modify CNSL into combination products. For preparing the combination product we will be using 10 % of CNSL and the rest 10 % will be substituted with the above mentioned essential oils by keeping the proportions of solvents and emulsifiers as such to produce two separate products. The properties of the formulations such as layer separation, sedimentation and creaming were evaluated visually.

Bloom test

The new formulations EC1 and EC2 were assessed by observing the bloom. 1 ml of the trial formulation was delivered to 99 ml of distilled water taken in 100 ml measuring cylinder by dipping the tip of the pipette 2 cm inside and emulsion bloom formed was observed and rated (Bessette, 2007).

Management of pests of bhindi using CNSL based botanical pesticides.

CNSL 20% EC along with combination products EC1 and EC2 developed were evaluated as botanical pesticides for bhindi pest management in a pot culture experiment using variety Anjitha. The crop was raised according to the KAU package of practices (KAU, 2016). All botanicals were evaluated at 1 %, the dose arrived at based on the results of laboratory experiment. Flubendiamide 39.35 SC @ 0.1%, Thiamethoxam 25% WG @ 0.03% and azadirachtin @ 0.03% served as checks. Water sprayed plants served as control. Two rounds of application one each at vegetative (35 DAP) and reproductive (58 DAP) stage were administered, and observations were taken before and after the treatments. The post treatment population of pests was observed on 1, 3, 5, 7 and 14 days after treatment. Phytotoxicity effects of different treatments were also evaluated.

Growth parameters of bhindi treated with different botanicals

The plant height and leaf number at both vegetative and reproductive stage (38, 49, 61 and 65 DAP) of the crop after the application of treatments were also recorded in the pot culture experiment to understand the effects of different treatments on plant growth. The number and weight (in grams) of the fruits were also recorded.

Statistical Analysis

Data on per cent mortality and mean population of pests in field were analysed by one-way analysis of variance (ANOVA) (Panse and Sukhatme, 1967) after arc sine and square root transformations, respectively using WASP 2.0 software (Web Assisted Statistical Package). Probit analysis was performed using the SPSS 16.0 version (Statistical Package for the Social Sciences).

Result and Discussion:-

The different concentrations of CNSL viz., 0.1, 0.25, 0.5, 0.75 and 1per cent, prepared from the CNSL 20 % EC formulation were evaluated against S. derogata under laboratory conditions. CNSL produced quick effect (1 DAT) on *S. derogata* evidenced by high mortality of 83.33 per cent @ 1 %. Larvae exposed to higher concentrations of CNSL developed black discolouration from first day after treatment whereas larvae treated with lower concentrations exhibited reduced feeding and dehydration, later turning black. Phenolic compound mediated cuticular tanning pathway responsible for production of melanin (Chapman, 2013) maybe the reason for development of black discolouration of larvae exposed to CNSL.

At 3, 5, 7 and 10 DAT, CNSL 1 % was found superior to rest of the CNSL treatments with 90 per cent mortality. Chemical check, flubendiamide 39.35 SC @ 0.1% recorded 100 per cent mortality. CNSL 0.1 %, 0.25 % and 0.5 % were on par with each other though inferior to CNSL 0.75%, CNSL 1% and chemical check. CNSL @ 1% which was found superior in the laboratory assay was selected for further evaluation. CNSL at higher concentration of 5 to 25 per cent was reported to cause 100 per cent mortality of the coconut rootgrub, L. coneophora (John, 2008). Though CNSL was proved to be effective at lower concentrations of 0.2 % and 0.3 %against sucking pests *A. gossypi* (Sundaran and Faizal, 2018) and *A. craccivora* (Lekha et al., 2020) a higher concentration was needed against chewing pests. In the present study a comparatively higher dose of 1per cent was required against *S. derogata*, a chewing pest of bhindi.

The dose mortality response was obtained after probit analysis. The LC_{50} and LC_{90} values of CNSL against S. derogata were 0.32 and 0.76 per cent respectively at 10 DAT. The LC_{50} value against *S. derogata* was comparable to that of *S. litura* (0.275) (Lekha, 2020) and *H. vigintioctopunctata* (0.268) (Deekshit, 2020). However LC_{90} value was much lower than that of *S. litura* (2.979) as well as *H. vigintioctopunctata* (4.35). From the probit analysis of the laboratory evaluation CNSL @ 1% was found to be the most effective treatment and was selected for further evaluation.

CNSL 20% EC was modified by substituting part of the active ingredient with botanical oils namely neem oil and pongam oil. The two combination products, EC1 (CNSL 10% + Neem oil 10%) and EC2 (CNSL 10 % + Pongam oil 10%) thus developed were evaluated for product stability as well as emulsion properties. The formulations were stable without any layer separation, sedimentation and creaming. The emulsion made from the formulations were rated based on properties like blooming and stability as per BIS standard (BIS, 1997). An excellent emulsion was obtained when EC2 was mixed with water (Score 5: White billowing emulsion, no droplets observed). Whereas a good emulsion was obtained when EC1 was mixed with water (Score 4: White billowing emulsion, very few droplets breaking away).

CNSL 20% EC along with combination products EC1 and EC2 developed in present study were evaluated as botanical pesticides for bhindi pest management in a pot culture experiment using variety Anjitha. All botanicals were evaluated at 1 %, the dose arrived at based on the results of laboratory experiment conducted using S. derogata as test insect. Flubendiamide 39.35 SC @ 0.1%, Thiamethoxam 25% WG @ 0.03% and azadirachtin @ 0.03% served as checks. Two rounds of application one each at vegetative (35 DAP) and reproductive (58 DAP) stage were administered and observations were taken before and after the treatments.

In vegetative stage three main pests were observed, viz leaf roller; *S. derogata*, jassid; *A. biguttula biguttula*and aphids; *A. gossyp*i the population which did not vary significantly before application of treatments.

In vegetative stage, at different intervals after treatment the lowest population of leaf roller, S. derogata was recorded in plants treated with Thiamethoxam 25 % WG @ 0.03%. At 1 DAT the result was on par with data recorded from plants treated with EC1 @ 1% (3.66 $plant^{-1}$), flubendiamide 39.35 SC @ 0.1% (4 $plant^{-1}$) and azadirachtin @ 0.03% (4.33 $plant^{-1}$). CNSL 2 % reported to cause 80 per cent mortality of L. coneophora (Jeevan and Sreekumar, 2015). Comparatively higher dose of CNSL was reported against S. litura also (Lekha, 2020). At 3, 5 and 7 DAT chemical check (Thiamethoxam 25 % WG @ 0.03%) was on par with EC1 @ 1%. Also EC1 @ 1% was on par with EC2 @ 1% at 7 DAT. So EC1 @ 1% and EC2 @ 1% were found to be most effective botanicals against leaf roller.

In the case of jassids,*A. biguttula biguttula* Thiamethoxam 25 % WG @ 0.03% (0-4.67 $plant^{-1}$) came out to be the most favourable treatment at all days after treatment. It was on par with flubendiamide 39.35 SC @ 0.1% (0-5 $plant^{-1}$), EC1 @ 1% (0.33-5.33 $plant^{-1}$) and azadirachtin @ 0.03% (0.33-6.33 $plant^{-1}$). Nitroguanidines viz; acetamiprid (20 g a.i/ ha) and thiamethoxam and imidachloprid (25 g a.i/ ha) proved most effective in controlling okra jassids (Acharya et al., 2002). EC2 @ 1% was found to be on par with EC1 @ 1% at 1 and 7 DAT. So EC1 @ 1% and EC2 @ 1% were found to be most effective botanicals against jassids. Spraying of botanicals like neem oil, neem seed, neem leaf extract, neem seed kernel extract against jassid were very successful (Thapa et al., 2019).

At 1, 5 and 7 DAT chemical control Thiamethoxam 25 % WG @ 0.03% (0-8.67 $plant^{-1}$) recorded the least population of aphids, *A. gossypi* and was on par with flubendiamide 39.35 SC @ 0.1% (0.67-9 $plant^{-1}$), EC1 @ 1% (0.67-9 $plant^{-1}$), azadirachtin @ 0.03% (0-2.67 $plant^{-1}$), CNSL 20% EC @ 1% (1.33-9.67 $plant^{-1}$) and EC2 @ 1% (1-

9 plant^{-1}). Also EC1 @ 1% was on par with EC2 @ 1% at 1, 3 and 5 DAT. So EC1 @ 1% and EC2 @ 1% were found to be most effective against aphids. The botanical insecticide azadirachtin was also found very effective against aphid, achieving more than 60per cent suppression (Ghosh, 2015).

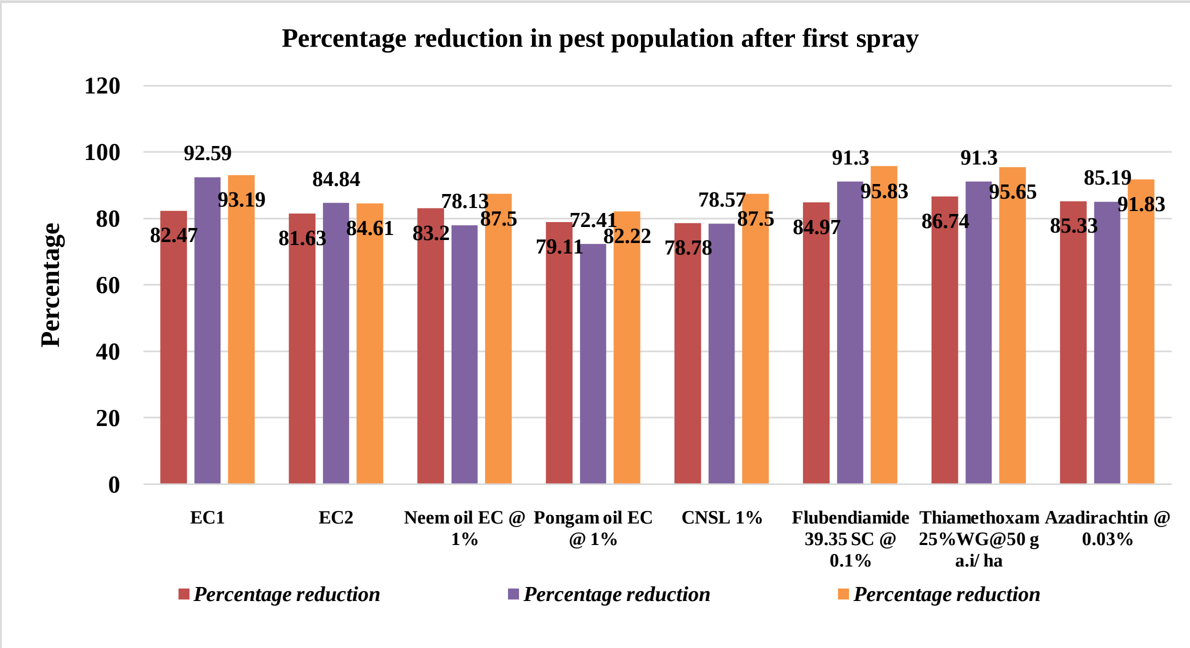

Fig 1:- Percentage reduction in pest population over control subsequent to botanical pesticide treatments after first spray.

Reduction in the population of various pests over control subsequent to botanical pesticide treatments was calculated and expressed in percentage and is shown in Fig 1. Both chemical checks (thiamethoxam 25 % WG @ 0.03% and flubendiamide 39.35 SC @ 0.1%, EC1 @ 1%) gave more than 90per cent control in the case of sucking pests (flubendiamide 39.35 SC @ 0.1% controlled 91.3per cent jasssids and 95.83 per cent aphids, thiamethoxam 25 % WG @ 0.03% controlled 91.3 per cent jassids and 95,65 per cent aphids) and more than 85per cent control for chewing pests (86.74 and 84.97per cent respectively). Population of pests on plants treated with EC1@ 1% was comparable to the percentage reduction recorded in plants treated with chemicals (Leaf roller: 84.47per cent, jassids: 92.59per cent, aphids: 93.19per cent). CNSL 1 % recorded 78.42 and 76.52 per cent reduction of white flies, *Bemisia tabacci* over control at 1 and 2 DAT, respectively and 100 per cent reduction at 3 DAT and CNSL 0.5 % and all the combination treatments shown 100 per cent reduction of aphids, *Aphis gossypi* over control (Deekhit, 2020).

In reproductive stage also the same three pests were observed. Pre-treatment population count was taken at 58 DAP and the population of all pests differed significantly. Plants treated with chemicals had the least population of the three major pests during pre-treatment count indicating persistent effect of first round of treatment. For aphids EC1 and EC2 gave long lasting effect in pest control.

Against leaf roller, during second round of application, thiamethoxam 25% WG @ 0.03% (0-1.33 plant^{-1}) was the most superior treatment and was on par with flubendiamide 39.35 SC @ 0.1% (0-1.67 plant^{-1}), azadirachtin @ 0.03% (0.33-2 plant^{-1}), EC1 @ 1% (0.33-2 plant^{-1}), EC2 @ 1% (0.67-2.33 plant^{-1}) and CNSL 20 % EC@ 1% (0.67-2.67 plant^{-1}). EC1 @ 1% and EC2 @ 1% were on par with each other. The best result given by a treatment containing CNSL was given by EC1 @ 1% and EC2 @ 1%.

Thiamethoxam 25 % WG @ 0.03% (0-1.67 plant^{-1}) and flubendiamide 39.35 SC @ 0.1% (0.33-2 plant^{-1})was found superior every day and recorded least population of jassids. At 5 and 7 DAT thiamethoxam 25 % WG @ 0.03% recorded 100per cent mortality. It was on par with flubendiamide 39.35 SC @ 0.1%, azadirachtin @ 0.03%, EC1 @ 1% and CNSL 1%. The best treatments containing CNSL were EC1 @ 1% and CNSL 1%.

At 1, 3 and 5 DAT flubendiamide 39.35 SC @ 0.1% (0-1.33 plant^{-1}) was recorded the best treatment for aphids and it was on par with EC1 @ 1% (0.33-2.33 plant^{-1}), CNSL 1% (0.67-3 plant^{-1}) and Thiamethoxam 25 % WG @ 0.03% (0.33-2.33 plant^{-1}). At 7 DAT 100 per cent mortality was observed in flubendiamide 39.35 SC @ 0.1% and azadirachtin @ 0.03%. The next best outcome was observed in thiamethoxam 25 % WG @ 0.03%, EC1 @ 1% and CNSL 1%. The best treatments containing CNSL were EC1 @ 1% and CNSL 1%. Larval mortality of S. obliqua recorded larval mortality of 20per cent till pupation upon treating with 1 per cent hydrogenated CNSL (Mahapatro, 2011).

Reduction in the population of various pests over control subsequent to botanical pesticide treatments during reproductive stage was calculated and expressed in percentage and is shown in Fig 2. Both chemical checks (thiamethoxam 25 % WG @ 0.03% and flubendiamide 39.35 SC @ 0.1%, EC1 @ 1%) gave more than 90per cent control in the case of sucking pests (flubendiamide 39.35 SC @ 0.1% controlled 92.86per cent jasssids and 100 per cent aphids, thiamethoxam 25 % WG @ 0.03% controlled 100 per cent jassids and 94.5 per cent aphids) and more than 80per cent control for chewing pests (100 and 81per cent respectively). Population of pests on plants treated with EC1@ 1% was comparable to the percentage reduction recorded in plants treated with chemicals (Leaf roller: 85.71 per cent, jassids: 95.46 per cent, aphids: 95.01 per cent). CNSL @ 3.7% produced 76.81 per cent of pest reduction over control of BPW, *O. longicolis* in leaf axil filling (Archa, 2020).

So it can be concluded that with EC1@ 1% and EC2@ 1% controlled the main pests in a rate comparable to that of chemical control and better than CNSL alone at the end of two sprays one each at vegetative and reproductive stage, respectively.Plants that received various treatments were observed for phytotoxicity symptoms if any following CIB&RC protocol. There was no notable phytotoxicity symptoms. However, CNSL at higher concentrations (4-5 %) was phytotoxic to brinjal plants and non-phytotoxic at lower concentrations (Deekshit, 2020). CNSL formulations were found to be safe in lower concentrations to cowpea (Lekha, 2019)

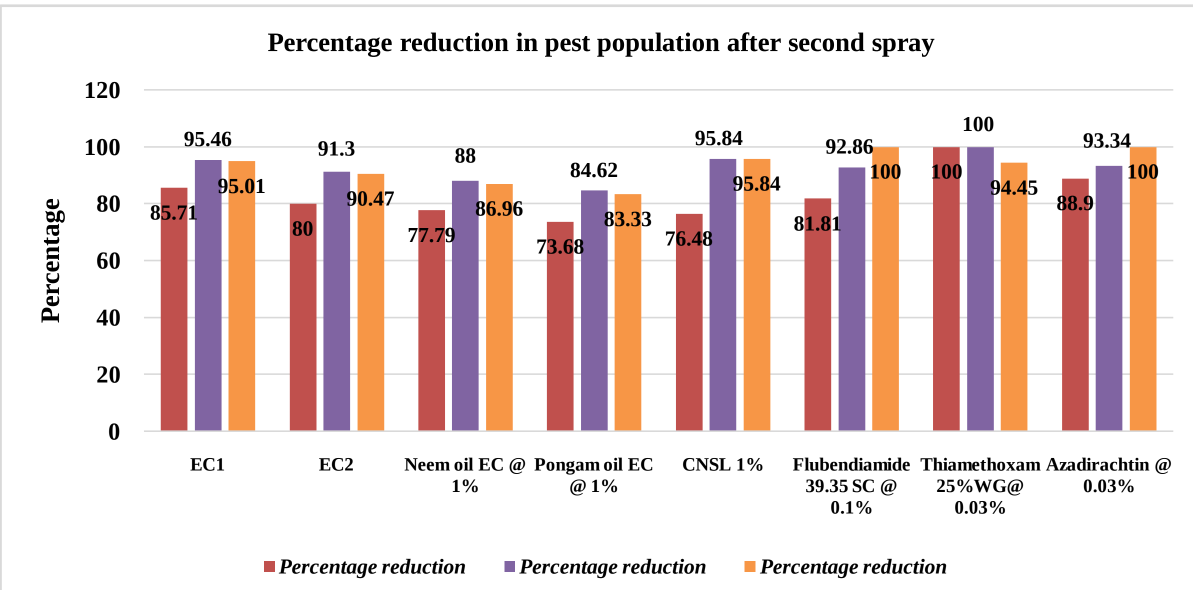

Fig 2:- Percentage reduction in pest population over control subsequent to botanical pesticide treatments after second spray.

The growth parameters like plant height and number of leaves were observed and evaluated to study the effects of CNSL on growth and yield at 3 and 14 DAT during vegetative stage and 3 and 7 DAT during reproductive stage. The data did not vary significantly for 3 DAT but did vary significantly at 14 DAT at vegetative stage. There was significant difference between plant height and leaf number during reproductive stage.

Plant height at 14 DAT in vegetative stage differed significantly and longest height was recorded for plants treated with flubendiamide 39.35 SC @ 0.1% (107.6 cm) it was on par with Thiamethoxam 25 % WG @ 0.03%,azadirachtin @ 0.03% and EC 1@ 1%. Shorter plants were observed under treatment with CNSL 20% EC @

1% (98.67 cm). During reproductive stage also plants treated with Thiamethoxam 25 % WG @ 0.03% (167.67 cm) were tallest and shortest height was recorded in plants treated with CNSL 20% EC @ 1% (160.83 cm). In vegetative stage, CNSL 0.25 % and CNSL 0.5 % recorded superior plant height in brinjal (Deekshit, 2020).

Mean of number of leaves during vegetative stage did not differ significantly during 3 DAT. At 14 DAT, plants treated with Thiamethoxam 25 % WG @ 0.03% (47.33) had the most number of leaves and were on par with flubendiamide 39.35 SC @ 0.1%. Least number of leaves was recorded in plants treated with CNSL 20% EC @ 1% (39.33). The same trend was observed in the reproductive stage also. The inability of CNSL treated chilli plants to reach growth proportionate to chemical treatments was earlier observed by Sundaran (2018). No such adverse effect was observed in cowpea (Lekha, 2020) and brinjal (Deekshit, 2020) and concentration 1per cent and below.No significant difference was observed in fruit number and the fruit yield under different treatments.

In the present study, CNSL 20% EC @ 1% could not produce growth proportional to chemical treatments. This limitation could be reduced by substitution of part of CNSL with neem oil and pongam oil as evidenced by the results obtained with combination product treatments.Thus based on the results of laboratory and field evaluation it was proved that the botanical pesticide combination formulations, EC1 and EC2, developed in the present study can be recommended for pest management in bhindi, at a dose of 1 % ai.

Conclusion:-

The results of the present study indicate that CNSL-based botanical pesticides can be an effective and sustainable alternative to synthetic pesticides for the management of pests of okra. Further studies are required to optimize the concentration and application method of CNSL-based botanical pesticides for different crops and pests. The use of botanical pesticides can promote sustainable agriculture and reduce the environmental and health hazards associated with synthetic pesticides.

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