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| **Evaluation of Locally Available Botanical Extracts for the Management of Cochineal (Dactylopius coccus C.) Insect in Eastern Amhara, Ethiopia** |  |
| *Zemenu Endalew1\*, and Gizachew Haylemariam2**1\*Adet Agricultural Research Center/ Woramit Horticultural Crop Research and Training Sub Center, P.O.Box 8, Bahir Dar, Ethiopia**2Sekota dry land Agricultural Research Center, P.O.Box 62, Sekota, Ethiopia**\*Corresponding author; E-mail:* *zemenu16@gmail.com* |
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|  |  | **ABSTRACT** |
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| **Received:** February 17, 2024**Revised:** May 30, 2024**Accepted:** June 19, 2024**Available online:** June 27, 2024 |  | *The plant cactus pear (Opuntia spp*.*) locally known as beles is the common perennial fruit crop in arid and semi-arid areas of northern Ethiopia and it is the most familiar and stable fruit crop. However, the cochineal (Dactylopius coccus) insect is the most devastating and dangerous insect pest that threatens the perennial cactus pear production in the northern part of the country. Hence, this research activity was carried out to evaluate different locally available botanical extracts against cochineal insects in hot spot areas of Waghimra administrative zone in 2021. Three botanical extracts (Bio admix, Neem, and Nicotina) with control were evaluated in purposively selected plots using RCB design with four replications. Extractions of bioactive botanicals were prepared by grinding leaves using a mortar and pestle. Results depicted that all botanical extracts which were tested under filed conditions were significantly reduced cochineal number and further population buildup compared to the control treatment. However, based on its accessibility and its ease of extraction techniques over the two botanical extracts on mortality rate (>60%) of cochineal insect, the tree tobacco (Nicotina gluaca) was very sizeable for the management of cochineal insect. It was also very cheap and accessible in the area for ease of preparation. Thus, following all extraction techniques and procedures, the use of 18g Nicotina per liter of water was commendable for the management of cactus pear cochineal insect in Eastern Amhara, Ethiopia, and related agroecology. Moreover, further studies on the biology, ecology, integrated management strategies, and chemical composition of botanicals should be conducted.*  |
| **Keywords:** *Botanicals, Cactus, Cochineal insect, Extraction methods, and Mortality rate*  |  |

1. **INTRODUCTION**

Opuntia, commonly called the prickly cactus pear is originated in the tropical and subtropical Americas and wild or cultivated, they can be found in a wide variety of agro-climatic conditions across the entire American continent. The plant has spread further through the people as they traded and settled in Africa, Asia, Europe, and Australia, where the cultivated and wild plants continue to provide food and materials (FAO, 2013, Adriana et al., 2014; Mondragón-Jacobo et al., 2001). Production and cultivation areas of prickly cactus pear species are mostly in marginalized areas due to its unique characteristics, which provide resilience to prolonged drought, desertification, and harsh environmental situations (Paolo et al., 2017). Cactus pear is able to grow on land where no other crops are able to grow; it can be used to restore degraded land and in many countries, such as Ethiopia, Morocco, South Africa, Kenya, India, Pakistan etc., it is the only crop that can be relied on when everything else fails (Paolo et al., 2017).

The introduction of the cactus plant to Northern Ethiopia is believed to have been between 1848 and 1870 through the Catholic missionaries who visited the Eastern zone of Tigray (Shushay, 2014). The cactus pear also locally called “Beles” or “Qulqual” is grown in arid and semiarid areas of Ethiopia and is characterized by drought-tolerant growth in erratic rainfall and poor soil conditions and can prevent soil erosion and enhance soil conservation (Mde Wal et al., 2015). It is highly cultivated and/or naturally grown in the Northern part of Ethiopia, and dominantly in Eastern Amhara Waghimra administrative zone including Tigray region.

In Waghimra administrative zone, the fruit cactus is the main food-secure crop from the beginning of summer to the end of summer i.e. from the first of June to the end of August. Regarding its health benefits, it contains low-calorie, saturated fat- and cholesterol-free sources of dietary fiber, vitamin C, calcium, and antioxidant compounds (Mostafa et al., 2014). Additionally, it is also used as an income source by selling the ripened fruit (Haftay et al., 2018). It also has medicinal value in treating several diseases and conditions, including diabetes, hypertension, hypercholesterolemia, rheumatic

pain, gastric mucosa diseases, and asthma (Ulises et al., 2014; Stintzing, 2005). However, the newly introduced cochineal (*D. coccus*) insect is a big challenge for cactus pear production. The insect was first introduced by Mekele University in collaboration with the FAO project for the purpose of carmine and fabric dye production and kept under a glass house (Yemane et al., 2020). The permission for field release was obtained from the federal government (ministry of agriculture), and then the insect was released to different locations on the 12th of September 2004 in Tigray, Ethiopia. The carmine dye is also used for different coloring purposes, food-stuff, cosmetics, and pharmaceuticals (Haftay et al., 2018). However, the insect was dispersed out to the surrounding cactus per production areas and its infestation was raised from 3.8ha to 75000ha and nowadays it causes substantial yield loss in cactus production (Yemane et al., 2020).

Cochineal insects (*D. coccus*) are soft-bodied, flat, oval-shaped scale insects. The females, remained immobile, wingless, and about 5 mm (0.2 in) long cluster on the cactus pad. They live in the form of colonies on the cladode of cactus and can suck and feed the juices by penetrating the cactus with their beaklike mouthparts (Liberato & Vigueras 2017). The female cochineal can hatch up to one thousand eggs after mating, and the fertilized females increase in size and give birth to tiny nymphs. The nymphs easily disperse via wind, animals, and other dispersal agents and can cover large areas within short time (Liberato & Vigueras 2017). The nymphs secrete a waxy white substance over their bodies for protection from predation, environmental stress, water loss, and excessive sun (Gaetana et al., 2018). That is why it is an environmentally persistent, highly devastating, and serious insect in cactus production areas of Waghimra zone and its infestation is almost in all cactus-growing areas. The plant is already endangered unless strict management strategies are designed. However, different scholars tried to manage this challengeable insect through various botanical extracts, insecticides and other organic pesticides.

According to Haftay et al. (2018) the indigenous plant locally named as Kotsili mariam is the most efficient botanical insecticides for controlling the insect cochineal and it had 100% mortality rate. Similarly, the botanical insecticide i.e. Neem oil (*Azadirachta indica*) extract and Nicotinia gluaca had high efficacy against the insect (Ibrahim et al., 2016). Even though botanical extracts like Neem (*Azadirachta indica*) and Tobacco (*Nicotina gluaca*.) are not common for the management of cochineal insect (*D. coccus*), intermittently these are used for the control of different insect pests such as aphid and flea beetles in Waghimra administrative zone (personal communication, 2021). Commercially available insecticides such as dimethoate 40% EC and karate 5% EC showed an above 70% mortality rate, which is very efficient against the expansion of cochineal insect in Tigray region, however, these products are very toxic both for human and honey bees and also not economical to recommended for use in edible fruits like cactus. Regarding Waghimra administrative zone, any management practice concerning to this newly introduced insect have not been done despite the infestation level is extremely very high. Therefore, we intend to evaluate promising botanical extracts and commercially available botanical insecticides, which were already tested in Tigray region. Thus, the objective focused on evaluating botanical extracts for the management of cochineal insect in Sekota zuria district.

1. **MATERIALS AND METHODS**
	1. **Description of the Study Area**

The experiment was conducted in the off-season of December 2020/21 under field conditions around Sekota dry-land agricultural research center. The area is located at 12⁰43ʹ57.2ʺN and 38⁰57ʹ52.7ʺ E with an elevation of 2122 meter above sea level and it is situated in the semi-arid agro-ecological zone of the region. The trial site is much marginalized and characterized by bushy shrub trees such as spiny *Acacia etbica,* cactus pear, and other related species. The rainfall pattern of the area is characterized by low and intermittent and its annual rainfall ranges from 554mm to 784mm while the temperature ranges from 15 to 29℃ (Kombolcha Metrology station, 2020).

* 1. **Raw Materials and Extraction Methods**

Plant materials used for the evaluation trial were collected from the surrounding and accessible areas of Waghimra zone. Leaves of true Neem tree were collected from Aberglle district and mature leaves of Tobacco tree (*Nicotina gluaca)* was collected from the surroundings of Sekota town. Commercially available anti cochineal bio admixture was bought from Mekelle. Then, the collected leaves were chopped and dried under shade condition for 7-10 days so as to maintain its active ingredient. Dried plant materials were grinding using mortar and pestle to produce a powder. Extracts of eighteen (18) gram of Nicotina gluaca and fifteen (15) gram of Neem powder were measured and mixed individually with one liter of water with the bench marks stated by the following scholars (Haftay et al., 2016; Ibrahim et al., 2016). In addition, 8ml kerosene per liter was added in both diluted botanicals for ease of penetration and loosen the hard white wax coverage of cochineal insect (Haftay et al., 2018). Squeezing and filtration using muslin cloth also proceed to separate the residue and extract. After mixing, shaking was accomplished to homogenize the mixtures. Finally, it was kept for 7 days for strong fermentation and chemical reaction.

* 1. **Experimental Design and Treatments**

The treatments were laid out in RCB design around the Sekota dry land agricultural research Centre using four localized and separated areas as replications. Each replication consists of four treatments with an area of 4m x 5m. The treatments were two botanical extracts (Neem and Nicotina spp.), a commercially available anti-cochineal botanical, and a non-spray control. The application rate of the bio-admixture was based on the manufacturer's recommendation of one liter of bio-admixture in fifteen liters of water, while Nicotina spp. and true Neem were using 18 g and 15 g per liter of water respectively (Table 1). Treatments were applied in the afternoon at seven-day intervals.

**Table 1:** List of treatments with their rate of application

|  |  |  |
| --- | --- | --- |
| Treatment code  | Full treatment name | Rate of application  |
| Bio add | Bio admixture | 1 litter per 15 liter water |
| Neem  | *Azadirachta indica* | 15g powder per 1 liter water |
| Nicot  | *Nicotina gluaca*  | 18g powder per 1 litter water |
| Con  | Control  |  |

NB: rate of botanical extract bench mark (Haftay et al., 2016; Ibrahim et al., 2016)

* 1. **Data collection and analysis**

The data were collected from 10 randomly selected and pre tagged cladode before spray, 24 hrs, 48 hrs, and 72 hrs after spray. A total of three times spray was done. Cochineal population during the period of study was recorded as indicator to the effectiveness of the applied treatments. The attached cochineal adults were counted from the sample cladode of cactus plant before and after treatment applications and expressed in mortality percentage rates. The total count data were subjected to mortality percentage and statistical analysis software (SAS, version 9.0) with mean comparison of 5% probability test. The count data before spray and mortality percentage data were transformed with square root transformation method.

1. **RESULTS AND DISCUSSION**

Evaluation and validation of botanical extraction and commercially available bio admixture were assessed with the benchmark comparison of untreated control treatments. The effects of

treatments on cochineal insect population and mortality rate were significantly high. Analysis of variance revealed that Nicotina (61%), Neem (39.7%) and bio admixture (56%) showed significantly higher mortality percentage of cochineal insect after 72 hours as compared to the control treatments (-12.5%). Negative value of mortality percentage indicates that cochineal insect population was significantly (p≤0.05) increased (Table 2). However, in the first spray of all treatments at 24 hours recorded, mortality percentage of cochineal insect was below 30% (Table 2). This might be due to unevenly distribution of botanical extracts while initial spraying time on webbed cochineal insect in both sides of cactus pear cladodes. Similarly the finding of (Ibrahim et al., 2016) reveals cochineal mortality percentage was significantly higher in the first spray of 72 hrs counting while 30% and below mortality percentage was recorded in 24 hrs of cochineal insect population.

**Table 2:** Mean comparison of treatments in their cochineal mortality percentage at 24 hrs, 48 hrs and 72 hrs after first spray of botanical extracts

|  |  |  |  |
| --- | --- | --- | --- |
| S/N | Treatments  | CPBS | Cochineal mortality % after 1st spray |
|  |  24 hrs | 48 hrs | 72 hrs |
| 1 | Bio admixture | 249(15.6) |  23.1(4.47) | 49.5(6.9) | 56.7(7.5) |
| 2 | Neem extract | 149.6(12.2) |  8.8(2.89) | 30.6(5.5) | 39.7(6.2) |
| 3 | Nicotina extract | 165.9(12.6) |  27.6(5.2) | 43.70(5.9) | 61.98(7.8) |
| 4 | Control | 167(12.7) |  1.7(0.81) | -10.8(1.7) | -12.5(1.7) |
|  | Mean  | 182.5 |  15.33 | 28.25 | 36.51 |
|  | CV (%) | 19.1 |  37.6 | 38.2 | 18.2 |
|  | LSD (0.05) | 4.78 |  2.36 | 3.61 | 1.99 |

CPBS = Cochineal population before spray, value in parenthesis are transformed data, CV = Coefficient of variation, LSD = Least significant difference, “Negative value” => indicates increment of cochineal population as compared to initial counting or before spray counting, CV and mean separation were obtained from transformed data

Botanical sprays have greatest impact on cochineal population reduction after 24 hrs, 48hrs and 72 hrs of counting in the second spray. Results indicated that application of bio admixture, Neem and Nicotina gluaca reveals significantly higher mortality percentage as compared to the control (Table 3). In spite of its insignificant difference, Neem extract showed higher cochineal mortality percentage in the second spray of all cochineal recordings. All the treatments except control have no any significant difference (p> 0.05) at 24 hrs, 48 hrs and 72 hrs of counting after spray (Table 3). This might be due to similar efficacy level of those botanical extracts on cochineal insect mortality rate. Similarly, different scholars depicted that most botanical extracts from indigenous plant species can have efficient control ability and greater mortality percentage (Haftay et al., 2018). In contrast to higher mortality percentage on the treated ones, least number of (1.8-5.2) mortality percentages was observed on unsprayed treatments. Despite its number is very low, the reason why mortality on unsprayed treatments might be attributed to various confounded factors such as intermittent predators, shell oldness, erratic rainfall shower and other microclimate variability. This was also agreed with the scholars (Chen et al., 2019) who states that, Changes in the frequency, duration, and intensity of erratic rainfall events are among the abiotic effects which may profoundly affect the development and survival of small organisms such as insects.

**Table 3:** Mean comparison of treatments in their cochineal mortality percentage at 24 hrs, 48 hrs, and 72 hrs after the second spray of botanical extracts

|  |  |  |  |
| --- | --- | --- | --- |
| S/N | Treatments | CPBS | Cochineal mortality % after 2nd spray |
|  |  24 hrs | 48 hrs | 72 hrs |
| 1 | Bio admixture | 120.7(10.7) |  40.6(6.3) | 42.8(6.5) | 61.1(7.8) |
| 2 | Neem extract | 89.2(9.4) |  60.7(7.7) | 56.7(7.5) | 76.7(8.7) |
| 3 | Nicotina extract | 75.8(8.6) |  48.2(4.9) | 49.6(7.1) | 54.1(7.3) |
| 4 | Control | 140(11.7) |  1.9(1.3) | 5.2(2.5) | 1.86(2.7) |
|  | Mean  | 106.4 |  37.85 | 38.59 | 48.43 |
|  | CV (%) | 17.42 |  11.1 | 20.2 | 16.9 |
|  | LSD (0.05) | 3.32 |  1.06 | 2.23 | 2.12 |

CPBS = Cochineal population before spray, CV = Coefficient of variation, LSD = Least significant difference, CV and mean separation were obtained from transformed data

Results indicated that plots sprayed with bio admixture, Neem, and Nicotina gluaca showed remarkable cochineal insect reduction as compared to the control. More than 86% mortality percentage of cochineal insect was recorded in all botanical treated cactus in the third spray of 24 hours counting (Table 4). In 48 and 72 hours of scouting, all botanicals (bio admix, Neem and Nicotina) botanical extraction depicted significantly higher (p<0.05) and comparable mortality percentage. Especially the botanical Nicotina gluaca had significant impact on cochineal population reduction in the three consecutive recordings despite of its insignificant difference from neem and bio admix botanicals with over advantage of its accessibility. In the third spray of 72 hours scouting, it reveals around 63% mortality (Table 4). According to (Ibrahim et al., 2016) the efficacy of Nicotina gluaca extracts on cactus pear cochineal insect was very high and it reveals around 93 % mortality rate at the second spray after 72 hours. Synthetic insecticides against cochineal insect are also comparable in efficacy or mortality rate with botanical extracts. This was also in agreement with the scholars (Shimat & Maria 2017), who stated that greenhouse tested insecticides had significantly high mortality rate comparing with untreated ones but among insecticides, there was insignificance difference. This implies that botanical extracts having insecticidal active ingredient and greater or comparable mortality percentage with synthetic insecticides were very pertinent due to their ease of affordability and soundness to the environment.

**Table 4:** Mean comparison of treatments in their cochineal mortality percentage at 24 hrs, 48 hrs, and 72 hrs after the third spray of botanical extracts

|  |  |  |  |
| --- | --- | --- | --- |
| S/N | Treatments  | CPBS | Cochineal mortality % after 3rd spray |
|  |  24 hrs | 48 hrs | 72 hrs |
| 1 | Bio admixture | 95.3(9.5) |  91.7(9.5) | 76.3(8.7) | 43.1(6.5) |
| 2 | Neem extract | 61.1(7.6) |  86.9(9.3) | 71.2(8.4) | 39.1(5.9) |
| 3 | Nicotina extract | 60.1(7.7) |  92.3(9.6) | 77.5(8.8) | 62.9(7.9) |
| 4 | Control | 123.2(11.1) |  1.6(1.2) | 4.4(2.6) | 4.4(2.6) |
|  | Mean  | 84.9 |  68.12 | 57.33 | 37.37 |
|  | CV (%) | 19.75 |  3.03 | 9.4 | 27.73 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | LSD (0.05) | 3.34 |  0.42 | 1.26 | 2.98 |

CPBS = Cochineal population before spray, CV = Coefficient of variation, LSD = Least significant difference, CV and mean separation were obtained from transformed data

As indicated in the graph above, the cochineal population was declined every 24 hours intervals counting after the application of Nicotina gluaca, Neem, and bio admixture botanical extracts. However, the population was slightly increased in the untreated cactus pear plant when it compared with the treated cactus plant (Figure 1).

**Figure 1:** Pulled cochineal population decrement due to botanical extract application every 24 hours of recordings

It is clear that the insect cochineal has already become a series, fast disseminated insect pest and consequences of total cactus pear damage without any remedial action in the region. However, the findings of this research gave some clues for managing cochineal insect in Waghimra administrative zone. The tested botanicals and bio admixture were promising enough in managing this quarantine (cochineal) pest. As indicated in the above graph, the insect cochineal populations were highly disturbed and failed its progress on the treated cactus pear cladode. The reason for the declining cochineal population might be due to the toxicity of botanical extracts against the newly pupated or crawlers’ stage of insects and even the newly emerged cactus cladodes were also free from any cochineal population after the final application of botanicals. This was also in agreement with (Ibrahim et al., 2016), which stated that plant extracts can produce a toxic substance (may be terpenoids) against the wild cochineal insects (*Dactylopius opuntiae*) especially it was toxic for the first and second instars of nymphs (crawlers) in cochineal population. Similarly different scholars such as (Adriana et al., 2014) reported that many botanical extracts of indigenous plants play a substantial role in reduced the number of cochineal crawlers by 71%.

However, cochineal (*Dactylopius opuntiae)* populations in the untreated cladode or control treatments were steadily increased since the cochineal population was unobstructed (Fig, 1). In addition, first and second instar nymphs (crawlers) of cochineal insect have easily invaded the new cladode of cactus and they form their protective shell against an external enemy and again recycle their fecundity so as to uphold its population. Thus, intensive and sustainable cochineal management with promising botanicals were a very good strategy for better cactus pear production.

1. **CONCLUSION AND RECOMMENDATION**

Results revealed that different botanical extracts, which were evaluated in Sekota zuria district showed the promising result, implies that the botanical extract Nicotina, Neem and bio admixture had a significant impact in reducing cochineal insect population. Above 86.9% mortality rate were observed because of Nicotina, Neem, and bio admix botanicals in the weekly interval and third phase of spray frequency after 24 hours. The contrarily maximum cochineal insect (140 insect/cladode) population were recorded on the treatments remains unsprayed. Hence Nicotina gluaca, Neem, and bio admix against cochineal insect were the most important management options in addition to their advantage in terms of environmentally friended nature. Additionally, uses of those three botanical extracts were very pertinent, especially in honey bee potential areas like Waghimra administrative zone and related agro ecologies. Though, the richness and accessibility of *Nicotina gluaca* in Waghimra zone is very high and its significant level in reducing cochineal insect was parallel to Neem and bio admixture botanicals. Thus, using *Nicotina gluaca* for the management of cochineal insect was commendable in Eastern Amhara, Ethiopia typically in Waghimra administrative zone of cactus pear production areas. Besides, further studies should be continued on its biology, ecology, and integrated management strategies even the chemical composition of botanicals in the study areas.

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